# Selecting and evaluating native forage mixtures for the mixed grass prairie

A Thesis Submitted to the College of

Graduate Studies and Research

in Partial Fulfillment of the Requirements

for the Degree of Master of Science

in the Department of Plant Science

University of Saskatchewan

Saskatoon

By

Jenalee Mischkolz

© Copyright Jenalee Mischkolz, April 2013. All rights reserved.

## Permission to use and disclaimer statement

In presenting this thesis in partial fulfillment of the requirements for a graduate degree from the University of Saskatchewan, I agree that the Libraries of this University may make it freely available for inspection. I further agree that permission for copying of this thesis in any manner, in whole or in part, for scholarly purposes may be granted by the professor who supervised my thesis work or, in their absence, by the Head of the Department or the Dean of the College in which my thesis work was done. It is understood that any copying or publication or use of this thesis or parts thereof for financial gain shall not be allowed without my written permission. It is also understood that due recognition shall be given to me and to the University of Saskatchewan in any scholarly use which may be made of any material in my thesis.

Requests for permission to copy or to make other use of material in this thesis in whole or part should be addressed to:

Head of the Department of Plant Sciences

University of Saskatchewan

51 Campus Drive

Saskatoon, Saskatchewan, S7N 5A8

**Acknowledgments**

Thank you to Dr. Eric Lamb, for his guidance, forethought, statistical expertise, and constructive criticism through the completion of the project, as well as providing me with numerous learning opportunities and skill sets. As members of my committee, I thank Dr. Michael Schellenberg for his support and mentorship in the field aspects, and writing process of this project, and Dr. Yuguang Bai for his attention to detail. Thank you to my grad chair Dr. Bruce Coulman, and a special thank you to my external examiner Dr. Bart Lardner.

Thank you to the forage research team, located at Agriculture and Agri-Food Canada's Semiarid Prairie Agricultural Research Center in Swift Current for their welcome, assisting with small plot work, and support throughout the study, including (but not limited to!): Jackie Bolton, Perry Coward, Melissa Kehler, and Russ Muri. Thank you to Jackie Bantle, and Eldon Siemens at the Agriculture Greenhouses for their very practical technical assistance. Additionally from the U of S Saskatchewan Plant Community Ecology Lab, I thank Sherri Friedrich, Amanda Guy, Brittany Letts, and Curtis Wiens for their assistance with data collection, and Christiane Catellier, Digit Guedo, Amanda Guy, and Michael Lavendar for their support, encouragement, and writing critiques throughout my studies. I thank my parents, Vanesa and Darald Mischkolz, and my friends for their support and encouragement during this venture in life.

This project was funded by a Beef Cattle Research Council grant to M. P. S. and E. G. L. and CFI Leaders Opportunity Fund and NSERC Discovery grants to E. G. L.

## Abstract

Diverse native seed mixtures have many benefits for prairie restoration or seeded pastures. In natural grasslands, species naturally coexist with hundreds of other species in complex communities. Commercial seed mixtures rarely contain more than a small number of species, often with haphazard ratios of the component species. Thus there is no natural template for combining selected species into an optimally productive community and there is limited knowledge on how to compose a suitable species mixture. Identifying which features of a community drive increased productivity may aid in screening species and community compositions, leading to mixtures that are more specifically designed to be stable, and highly productive for the region. There is renewed interest native species as they have the potential to provide non-invasive, productive, and drought resistant rangelands that may prove more sustainable. Seven species with high agronomic potential and a broad native geographic distribution were selected for testing including: nodding brome [*Bromus anomalus* (Coult.)], blue bunch wheatgrass [*Pseudoregneria spicata* (Pursh)], western wheatgrass [*Pascopyrum smithii* (Rydb.)], side oats grama [*Bouteloua curtipendula* (Michx.)], little blue stem [*Schizachyrium scoparium* (Michx.)], purple prairie clover [*Dalea purpurea* (Vent.)], and white prairie clover [*Dalea candida* (Willd.)].

The early productivity and nutritional quality of these species was determined in simple mixtures in two field sites: Saskatoon and Swift Current. In the field sites the mixtures included all seven monocultures, 21 two-species mixtures and a mixture with all species. Productivity may be driven by the species richness, functional group richness, and species evenness of the community, the abundance and occurrence of particular species or functional groups, and average plant trait values within the community. Therefore, identifying the features of a

community that drive increased productivity and applying them as predictive tools may aid in screening species and community compositions. Many complex mixtures of the species were planted in greenhouse experiments to determine the strongest drivers of productivity for communities of these species. The experimental approach was validated in a confirmatory experiment where optimum communities were tested. These results did not differ under a moderate drought treatment. Results were generally consistent between field and greenhouse studies. Western wheatgrass (WWG) had the highest overall plant density and the strongest effect on the forage yield of the mixtures and communities. In the field study, productivity and crude protein content were not reduced when other species were also included with WWG in the mixture. *Dalea* spp. did not establish as well as the other species, but had the highest crude protein concentrations. The strongest predictors of productivity were the presence and abundance of perennial $C_3$ grasses. Increases in species richness, functional group richness, and the presence of $C_3$s (more specifically western wheatgrass) also increased productivity, likely because of the high early relative growth rate and strong competitive ability of western wheatgrass. Overall, communities screened in the greenhouse reflected early establishment field results. The systematic approach for evaluating communities can be modified to consider enhancing other ecological functions in addition to high productivity, in other regions.

## Table of Contents

## List of Tables

## List of Figures

# 1 GENERAL INTRODUCTION

## 1.1 Advantages of native species

There is a growing interest in the use of native perennial species for seeded rangeland and reclamation following disturbance as native species may increase carbon sequestration, improve wildlife habitat, lower agronomic inputs, and extend the grazing season (Wilson and Gerry 1995; Harper-Lore 1996; May et al. 1998; Richards et al. 1998; Schellenberg et al. 1999; Symstad et al. 2003; Jefferson et al. 2004; Petersen et al. 2004; Jefferson et al. 2005; Prairie Conservation Action Plan Partnership 2005; Moncada et al. 2007; Weigelt et al. 2009; Simmers and Galatowitsch 2010; Tracy et al. 2010; Muir et al. 2011; Thorne and Cardina 2011; Schellenberg et al. 2012). Native mixtures have the potential to be as productive as tame monocultures over a greater range of environmental conditions (Schellenberg 2008). Species that are adapted to an area or "ecotypes" may have increased tolerance to local climate and diseases, as well as positive associations with mycorrhizae and pollinators (Dorner 2002). Tolerance to environmental stress, specifically drought, is important in semi-arid grasslands, where plant growth is limited by water availability (Herbel et al. 1972; Tilman and Elhaddi 1992; Johnson and Asay 1993). Locally adapted seed may increase the establishment and propagation success (Knapp and Rice 1997). Ecologically adapted seed may be obtained from similar habitats if locally adapted seed cannot be used due to limited seed availability or economics (Jefferson et al. 2002); however, using populations from different regions may result in poor establishment and productivity (Kilcher and Looman 1983). Efforts to increase locally adapted native seed availability and use are being made by organizations such as the Native Plant Society of Saskatchewan, Ducks Unlimited, and Agriculture and Agri-Food Canada.

## 1.1 Plant interactions, productivity, and community structure

Productivity is linked to the type and intensity of interactions between plants in a community where plants may either compete or complement each other in regard to resource acquisition and growth (Trenbath 1974a; Miller 1994; Hooper et al. 2005; Sheehan et al. 2006; Lamb et al. 2011). Productivity has been shown to increase with diversity, along with increased resource capture, nutrient recycling, and stability (for a review see: Loreau and de Mazancourt 2013). The productivity of a complex community can be compared to monoculture productivity because monocultures can be assumed to be a baseline for each species' potential productivity in a given environment.

If shoot production (above ground biomass) in a mixture is larger than that expected from monocultures, the combination is overyielding (Trenbath 1974a) but see Garnier et al. (1997). This is evidence for the occurrence of positive interactions within the community. Positive or complimentary interactions between plants are due to combinations of traits or functional roles that are beneficial and can increase productivity; complimentarity is most evident when species possess different traits (Hooper et al. 2005; Brooker et al. 2008). Communities that overyield contain species that complement each other in their resource use through the occupation of different niches or through facilitation (Callaway 1995; Tracy and Sanderson 2004; Brooker et al. 2008). Niche differentiation can be in regards to the type of resource required, temporal use of resources (i.e. timing of resource use or productivity), or spatial use of resources (i.e. occupying different layers in the soil or canopy) (Tracy and Sanderson 2004). Facilitation is another form of complimentarity where the benefits of the interactions are not equal for both plants. For example, low soil nitrogen availability may be detrimental to grasses, but can be alleviated in the presence of legumes, (Bertness and Callaway 1994; Tilman 1997; Bruno et al. 2003; Muir et al.

2011) whereas the grasses may not have a strong positive influence on the legumes. Facilitation may be critical to maintain productive communities in severe environments (Choler et al. 2001; but see Maestre et al. 2005; Callaway 2007).

Negative interactions, or competition, between plants may reduce the forage yield of a mixture of species causing underyielding compared to monocultures (Trenbath 1974b), which may be a result of the relative competitive abilities of the plants. The competitive ability of a plant has two components, the ability to endure growth suppression by neighbours (competitive response; CR), and the ability to suppress neighbours (competitive effect; CE) (Goldberg et al. 1990; Goldberg 1996). Competitive effect ability is linked to size related plant traits, such as relative growth rates in high nutrient conditions, and root-related traits, such as specific root length in low nutrient conditions. In contrast, competitive response ability is a function of plant traits related to a plant's ability to persist and avoid damage from neighbours (Wang et al. 2010).

Optimum forage swards with higher productivity may be found in diverse plant mixtures where complimentarity occurs and competition is minimized (Brooker et al. 2008). Many studies have shown that increased diversity is associated with increased productivity (Wight and White 1974; Walker 1995; Tilman 1996; Walker et al. 1999; Lehman and Tilman 2000a; Díaz and Cabido 2001; Tilman et al. 2001; Reich et al. 2004; Hooper et al. 2005; Balvanera et al. 2006; Sheehan et al. 2006; Kirwan et al. 2007; Weigelt et al. 2009). Components of diversity include species richness, functional group richness, and species evenness of the community, species and functional group composition, and/or community-wide averages of plant traits such as relative growth rate (RGR), specific leaf area (SLA), specific root length (SRL), and competitive ability. There is still much debate, however, on which components of diversity are most closely linked with productivity.

### *1.1.1 Species Richness*

Increases in species richness can increase productivity (Tilman 1996; Lehman and Tilman 2000a; Tilman et al. 2001; Kirwan et al. 2007). High species richness in communities can be beneficial because species possess different traits and as more traits become present it can lead to increased functionality of the ecosystem (Weigelt et al. 2009). Additionally, as species richness increases, traits will become more prevalent resulting in functionally redundant species in the community. Functional redundancy has been shown to increase stability because the loss of a species may not result in the loss of a trait (Hooper et al. 2005). Additionally, with increased species richness there is increased probability of including a highly productive species, also known as the selection effect.

### *1.1.2 Relative abundance*

Increased relative abundance (evenness) in the community may also increase complimentarity among component species and thus productivity in the community (Lamb et al. 2011). Evenness is a measure of diversity because the evenness of the community determines the number of interactions between species which may be beneficial or detrimental (Sheehan et al. 2006). Relative abundance may be as important as species richness as a measure of diversity because the influence of additional species at low abundance may be small. For example, a community may effectively function as a monoculture if it 2 of the species have low abundances despite a species richness of 3 (Kirwan et al. 2007).

### *1.1.3 Functional group richness*

Species may be grouped in terms of their functional role in the community. Here we define functional groups in terms of the ecological significance as $C_3$ grasses, $C_4$ grasses, and legumes, although other classification systems have been suggested (Díaz and Cabido 2001; Morgan et al. 2001; Craine et al. 2002; Hooper et al. 2005). Increases in the number of functional roles in the community, or functional group richness, can also increase productivity (Reich et al. 2004; Balvanera et al. 2006). Some species may be functionally redundant to the system as multiple species perform the same role (Walker 1995; Walker et al. 1999; Díaz and Cabido 2001).

### *1.1.4 Plant traits*

The traits possessed by individual plants in a community can be averaged to a community-level value for each trait. Traits of interest include relative growth rate (RGR), specific leaf area (SLA), and specific root length (SRL). A high RGR may indicate that a species has a competitive life history strategy, adapted for maintaining dominance in high nutrient, low stress conditions. Conversely, a lower RGR may correspond to a more stress-tolerant life history strategy which is beneficial in nutrient deficient or otherwise stressful habitats (Grime 1974; Grime and Hunt 1975). SLA is an indication of investment of carbon in leaves. A low SLA corresponds to a species with small thick leaves that require high investment relative to photosynthetic ability; conversely plants with high SLA have large thin leaves that require less investment. High SLA is correlated with high RGR, large broad leaves, and aggressive, fast growing plants are generally more palatable and resilient to grazing (Grime 1977). High SRL is associated with increased nutrient and water acquisition ability (Aerts et al. 1991). SRL is a highly variable trait among species, and a plant may adapt to compete in the presence of other plants by altering the

arrangement of roots or foraging strategies (but see Cahill 2003; Weigelt et al. 2005). The ability of a species to increase SRL in response to competition may also allow higher productivity.

## 1.2 Advantages of diverse rangelands

Maintaining forage quality and quantity is challenging but necessary in grazing systems where herbivory and/or drought stresses plants (Heady and Child 1994; Gerrish 2001). Mixtures of species are expected to provide more stable production over the growing season and across years than monocultures because fluctuations in individual species' forage yield would be compensated by those of other species in the community (Doak et al. 1998; Tilman 1999; Lhomme and Winkel 2002). More diverse mixtures may provide a more reliable source of forage yield even in years with very different environmental conditions (Wight and White 1974; Lehman and Tilman 2000b; Schellenberg 2008). Compensatory dynamics in mixtures occur when the relative abundance of one species oscillates with the relative abundance of another (Grman et al. 2010; Roscher et al. 2011). Drought tolerant $C_4$ grasses, for example, may compensate for $C_3$ grasses which become dormant in hot, dry conditions. Thus, mixtures of native perennial species have high potential for long-term production stability despite environmental variability because diversity increases the reliability that the community will have long-term average performance (Yachi and Loreau 1999).

### *1.2.1 $C_3$ vs. $C_4$ grasses*

In native dry mixed-grasslands, $C_3$ and $C_4$ grasses are the dominant producers of biomass. Typically, grasses provide most of the digestible energy with $C_3$ grasses, the dominant group, producing the bulk of the forage yield (Schellenberg and Banerjee 2002; Muir et al. 2011). $C_3$

grasses grow early in the cool season and are dormant later in the growing season, whereas $C_4$ grasses initiate growth later in the season but continue growing through the summer (Cooke 1972; Lehman and Tilman 2000b; Tilman et al. 2001; McGraw et al. 2004; Schellenberg et al. 2012). This temporal division in new nutritious biomass can help to maintain forage yield and quality throughout the season.

### *1.2.2 Legumes*

Legumes fix atmospheric nitrogen into the soil; if released through mineralization the nitrogen can facilitate grass yield and quality, and reduce fertilizer requirements in nitrogen deficient rangeland systems (Callaway 1995; Oelmann et al. 2007; Temperton et al. 2007; Brooker et al. 2008; Whitbread et al. 2009; Muir et al. 2011). Including legumes (both tame and native) is beneficial to shrub mixtures as well (Schellenberg and Banerjee 2002). Furthermore, legumes have high protein concentrations which are useful for forage swards (McGraw et al. 2004; Muir et al. 2011).

### *1.2.3 Rangeland forage nutrition*

Crude protein concentrations can be used as an indicator of forage quality (McGraw et al. 2004), although neutral detergent fiber (NDF; a measure of the amount of structural fiber in the plant), and acid detergent fiber (ADF; a measure of cellulose and lignin in the plant) are also commonly reported as they are associated with digestibility (Van Soest 1982). Crude protein concentrations are highest in young plant tissue and decline as plants mature (Muir et al. 2011). Including $C_4$ and legume species is an important means to increase crude protein in a forage sward. Crude protein concentrations differ between the functional groups in the growing season. $C_3$ grasses provide high nutritional quality early in the growing season, whereas, $C_4$ grasses and legumes

provide high nutritional quality later in the growing season. Additionally, grasses cure better than legumes and therefore provide more crude protein in the fall (Buxton 1996; Holechek et al. 2004). Legumes, however, have higher protein concentration than grasses (Cooke 1972; Schellenberg and Banerjee 2002; McGraw et al. 2004). Therefore, a mixture of the functional groups may supply higher sequential crude protein concentrations over the growing season.

## 1.3 Rangeland species of interest

In Saskatchewan, the Mixed Grassland or Mixed Grass Prairie ecoregion (Padbury et al. 1998) is located within the Prairie ecozone (24 096 600 Ha) and is similar to the dry brown soil zone described previously (Abouguendia 1990). This ecoregion is divided into moist mixed grassland, and dry mixed grassland ecosites, where the moisture indexes (annual precipitation minus annual potential evapotranspiration) are <-325, and -325 to -225 mm, respectively (Hogg 1994). Bruynooghe and Macdonald (2008) defined five community types within the Mixed Grass Prairie in Saskatchewan, including: Blue Grama/Wheatgrass, Needle-and-Thread/Blue Grama, Needlegrass/Blue Grama/Wheatgrass, Wheatgrass/June Grass, and Needle-and-Thread /Wheatgrass (Western Porcupine grass/Northern Wheatgrass). In the Mixed Grassland ecoregion only 31% of native dominated grassland remains, largely due to conversion to cropland (Bruynooghe and Macdonald 2008). The Mixed Grassland in Saskatchewan is heavily used as a source of cattle production with a total of 9 308 437 ha of land dedicated to forage with 5 175 789 as native range, and 1 962 222 to seeded rangeland (Saskatchewan Forage Council 2010).

Re-establishing native species can restore ecological integrity as species can be more resistant to local conditions through evolutionary adaptation (Richards et al. 1998). The species chosen for this study originated in Canadian mixed grasslands, and thus should be better able to

cope with stress associated with this dry ecosystem than the non-native species commonly used as seeded pastures. Species that were selected for evaluation have high agronomic potential (reasonable: plant size, plant nutritional quality, cost, availability, and the ability to work with conventional machinery). Additionally, they have broad native geographic range spanning western Canada, and are currently being evaluated in various breeding programs located in Saskatoon, Swift Current, and Lethbridge. However, these species naturally coexist with hundreds of other species in complex communities and the selected species are not necessarily present in any given community. There is no natural template for combining these seven species into an optimal community. This unique combination of species may have the potential to provide stable, highly productive plant communities across Saskatchewan, despite a variety of stresses. Native species are notoriously difficult to establish and manage compared to domestic species, and complete stand failure has occurred (i.e. Bement et al. 1965; Wilson and Gerry 1995). Therefore patience, and more long term research, is needed to fully understand the potential of native perennial species for use as forage swards.

The selected species are: *Pascopyrum smithii* [Rydb.] (Western Wheatgrass, WWG), *Pseudoregeneria spicatum* [Pursh] (Bluebunch Wheatgrass, BWG), *Schizachyrium scoparius* [Michx.] (Little Blue Stem, LBS), *Bouteloua curtipendula* [Michx.] (Side Oat Grama, SOG), *Bromus anomalus* [Coult.] (Nodding Brome, B), *Dalea purpurea* [Vent.] (Purple Prairie Clover, PPC), and *Dalea candida* [Willd.] (White Prairie Clover, WPC). Many other native species could have been evaluated including but not limited to needle-and-thread (*Stipa comata*), june grass (*Koeleria macrantha*), western porcupine grass (*Stipa curtiseta),* sand dropseed (*Sporobolus cryptandrus),* thread-leaved sedge (*Carex filifolia*), winterfat (*Krascheinnikovia lanata),* Nuttall's saltbush (*Atriplex gardneri*), blue grama (*Bouteloua gracilis*), Sandberg's

bluegrass (*Poa sandbergii*), plains reed grass (*Calamagrostis montanensis*), prairie muhly (*Muhlenbergia cuspidate*), pasture sage (*Artemisia ludoviciana*), moss phlox (*Phlox subulata*), broomweed (*Gutierrezia sarthrae*), rose (*Rosa acicularis*), cactus (*Optuntia spp.*), rough fescue (*Festuca scabrella*), green needlegrass (*Stipa viridula*), northern wheatgrass (*Agropyron dasystachyum*), sedges (*Carex spp.*), crocus (*Anemone patens*), aster, yarrow (*Achillea millefolium*), Canada bluegrass (*Poa compressa*), and western snowberry (*Symphoricarpos occidentalis*). The following sections describe the biology of the seven native, perennial species selected for evaluation.

### *1.3.1 Western wheatgrass (WWG)*

Western wheatgrass [*Pascopyrum smithii* (Rydb.)] is a perennial, cool-season grass native throughout most of North America (Hitchcock 1971; United States Department of Agriculture National Resources Conservation Service Plant Materials Program 2002b; Monsen et al. 2004; Bruynooghe and Macdonald 2008; Stubbendieck et al. 2011). It has strong uniform sod-forming rhizomes, and generally shallow roots (<25 cm) (Monsen et al. 2004). The species is tolerant to alkaline, saline, and sandy soils, but does best on clay soils in lowlands (Monsen et al. 2004; Bruynooghe and Macdonald 2008). It is more drought tolerant than its non-native forage counterpart, crested wheatgrass (Frank 1994), and does not have the negative connotations associated with exotic invasive species. Western wheatgrass stands have been shown to be resistant to invasion from weeds and exotic species (Knowles 1987; Gillen and Berg 2005). WWG has scaborous, stiff, flat, blue-green leaves that grow at 45 degree angles from the stem. Its leaves have prominent veins on the upper surface, large purple auricles, and an inconspicuous ligule (Stubbendieck et al. 2011). WWG's seed spike is erect and approximately 3-6 inches long with 1 or 2 spikelets at each node.

Western wheatgrass has high potential for a native perennial forage sward. It begins growing early in spring, flowers in June, and matures late in the season (Bruynooghe and Macdonald 2008; Stubbendieck et al. 2011). WWG produces most of its available forage yield from mid-May to the end of June (Schellenberg et al. 1999). Yields for WWG are reported as variable and dependant on moisture conditions. For instance: Knowles (1987) reported a yield of 1919 kg/ha in Saskatoon, whereas in North Dakota Hofmann (1993) reported 3520 kg/ha and Karn et al. (1999) reported 5018 kg/ha. WWG is palatable, digestible, cures well on the stem, and has sufficient nutritional quality with 6.6 % digestible protein content (Cook et al. 1956) to maintain a dry beef cow in the second trimester of pregnancy (Jefferson et al. 2004; Monsen et al. 2004; Bruynooghe and Macdonald 2008).

### *1.3.2 Blue bunch wheatgrass (BWG)*

Blue bunch wheatgrass [*Pseudoregneria spicata* (Pursh)] Scribn. & Smith, previously "*Agropyron spicatum*", is a perennial, cool season non-rhizomatous bunch grass native to western North America (Hitchcock 1971; Miller et al. 1986; Monsen et al. 2004; Stubbendieck et al. 2011). BWG occurs in arid to semi-arid regions on a variety of soils, including coarse mountain slopes or dry calcareous soils, but is not tolerant to alkaline or saline soils. It has coarse fibrous roots that can be very deep rooted (4 feet) with many short laterals (Weaver 1919). BWG leaves have auricles and ligules that are 1 mm long. Its seed spike is slender and approximately 6-20 cm long with 1 spikelet per node (Stubbendieck et al. 2011).

BWG begins growing in early spring, enters dormancy in mid July, and can provide all season forage (Weaver 1919; Harris 1967; Stubbendieck et al. 2011). It requires careful monitoring to prevent overgrazing, particularly if grazed intensively or during late spring (Daubenmire 1940; Miller et al. 1986). Blue bunch wheatgrass swards productivity and crude

protein can be highly variable, depending on site conditions. Wilson et al. (1966) found yield of 583 kg/ha and 415 kg/ha in "good" and "poor" sites respectively in Washington, whereas, Mason and Miltimore (1959) reported 717 kg/ha in British Columbia. Blue bunch wheatgrass is palatable, and cures well. Peek (2010) found a 10 year average biomass of 393 kg/ha in central Idaho, and a protein concentration of 6.5% (6.25 * nitrogen %) at an elevation of 1244 m. Mason and Miltimore (1959) found BWG had a protein content of 3.9 %. Alternately, Ganskopp (2004) reported that a blue bunch dominated community in Oregon had a crude protein content of 3.1 %. In a review Miller (1986) concluded that BWG's forage quality was "sufficient for lactating animals during early spring but decline[d] markedly after flowering".

### *1.3.3 Nodding brome (B)*

Nodding brome [*Bromus anomalus* (Coult.)] is a perennial, cool-season, non-rhizomatous bunch grass that is native to western North America (Hitchcock 1971; Monsen et al. 2004). The grass grows best on dry to moderately dry soils in open communities at lower elevations. B has pubescent, flat, and broad leaves with small, irregularly toothed ligules and no auricles (Monsen et al. 2004). Its panicles are 7-14 cm long, open, and nodding.

The use of *Bromus* species in pastures has been largely restricted to *B. inermis*, a widely used introduced species (May et al. 1998). The success of *B. inermis* raised interest in a native perennial alternative, *B. anomalus*. May et al. assessed *B. anomalus's* establishment and biomass for three years, and crude protein content in two years at sites in western Canada. They reported high values for both biomass and crude protein where *B. anomalus* had approximately half of the biomass and equal forage quality of *B. inermis* (*B. anomalus* biomass = 4400 kg/ha; crude protein = 15%).

### *1.3.4 Side oats grama (SOG)*

Side oats grama [*Bouteloua curtipendula* (Michx.)] is a perennial, warm-season tuft grass with sod forming rhizomes (Monsen et al. 2004; Stubbendieck et al. 2011). It is native to most of North America, growing best on southern mixed prairies, but also on plains and foothills (Hitchcock 1971). SOG is found on a variety of soils but is better adapted to alkaline than acidic conditions (Nicholson and Bonham 1977; Stubbendieck et al. 2011). SOG is very drought resistant, likely as it does not rely on surface moisture availability and continues growing in drought conditions due to its deep, highly branched roots (5.5 feet) (Weaver 1958; Monsen et al. 2004). It is the largest *Bouteloua* species, growing between 0.2-1m tall. SOG's leaves are light green and flat, with long hairs scattered on both surfaces (Stubbendieck et al. 2011). Its ligules are 1 mm long, lacerate, and ciliate, and it does not have auricles. SOG's stems are slender, erect, and purplish. The species name arose from its seed head which as a panicle 14-20cm long with 25 to 80 short stalked branches that produce 2-8 spikelets along one side of the seed head (Monsen et al. 2004; Stubbendieck et al. 2011).

There is limited information on the forage yield or crude protein content of SOG despite it being thought by some to be the most promising *Bouteloua* for domestication (Hitchcock 1971; White 1986). This $C_4$ species produces green herbage in spring, but palatability is greatest in the summer and fall, and declines in winter (White 1986; Stubbendieck et al. 2011). White (1986) found the average forage yield in Montana was 980 kg/ha over four years with 9% crude protein.

### *1.3.5 Little blue stem (LBS)*

Little blue stem [*Schizachyrium scoparium* (Michx.)] is a perennial, warm-season, bunchgrass native throughout most of North America, most commonly found in the tall grass prairie

(Hitchcock 1971; United States Department of Agriculture National Resources Conservation Service Plant Materials Program 2002b; Bruynooghe and Macdonald 2008; Stubbendieck et al. 2011). LBS's roots are highly plastic and range from 2.4 m in sand hills to 0.3 m in a clay or silt loam, with a maximum lateral spread of 0.9 m (Weaver 1958). This species grows up to 60 cm tall. LBS's leaves are flat to folded, short along the stem, and keeled with a purplish base (Stubbendieck et al. 2011). It has ligules that are 2 mm long, membranous and ciliate, and does not have auricles (Bruynooghe and Macdonald 2008; Stubbendieck et al. 2011). Characteristic of this species, a branched panicle on each stem produces awned seeds that are covered in fluffy hair.

Little bluestem is palatable, providing fair to good forage, is an increaser species under moderate grazing, but suffers under intensive grazing (Bruynooghe and Macdonald 2008; Jackson et al. 2010; Stubbendieck et al. 2011). There is limited information on the agronomic potential of this species, however Phan and Smith (2000) found that LBS seed production was highly variable in Manitoba with the best performance in more southern locations. Initial establishment was reported to be low (Robins et al. 2009). White (1986) also evaluated LBS in Montana and found the average forage yield for four years after allowing it to establish for two years was 840 kg/ha with 7% crude protein. Conversely, it had a crude protein concentration of 13% in Alabama (Powel et al. 2003).

### *1.3.6 Purple prairie clover (PPC)*

Purple prairie clover [*Dalea purpurea* (Vent.)] is a warm season legume native to central North America (United States Department of Agriculture National Resources Conservation Service Plant Materials Program 2002a; Tannas 2004; Stubbendieck et al. 2011). It grows to a height of 30-90 cm tall with leaves are divided into 3-5 narrow leaflets, and a deep taproot. The species

has a dense cylindrical spike of purple flowers which mature acropetally (Stubbendieck et al. 2011).

PPC has been used in restoration seed mixtures because it is drought tolerant, however it is slow to establish with relatively low forage yield (Schellenberg and Banerjee 2002; McGraw et al. 2004; Molano-Flores et al. 2011). It has high protein, low fibre content, and is rated as a fair forage sward that increases under moderate grazing but suffers when overgrazed (Schellenberg and Banerjee 2002; McGraw et al. 2004; Tannas 2004; Stubbendieck et al. 2011). Schellenberg and Banerjee (2002) found PPC has a protein concentration of 18%, while McGraw et al. (2004) found 15.2%. McGraw et al. (2004) also found it was more digestible (had lower ADF) than commonly used introduced forage legumes. Jin et al. (2012) determined that including PPC in a ruminants diet may improve health due to antimicrobial properties.

### *1.3.7 White prairie clover (WPC)*

White prairie clover [*Dalea candida* (Willd.)] is a warm season legume very similar to PPC and native to central North America (United States Department of Agriculture National Resources Conservation Service Plant Materials Program 2002a; Tannas 2004). It grows to a height of 30-90 cm tall with leaves divided into 5-9 oblong-oblanceolate leaflets, and a deep taproot. The species has a dense cylindrical spike of white flowers which mature acropetally.

Limited information on this species agronomic potential is available. McGraw et al. (2004) found that WPC had low forage yield but good forage quality, with a protein concentration of 12.7%. Additionally, they found it was more digestible (had lower ADF) than commonly used introduced forage legumes.

## 1.4 Thesis Objectives

In natural grasslands, numerous species naturally coexist in complex communities. Seed mixtures for restoration or seeded rangeland rarely contain all possible species and commercial seed mixtures targeted for restoration have haphazard ratios of selected species. Though species selected for a given mixture may be native to a region, all species are not necessarily present in any given site. Thus there is no natural template for combining the selected species into an optimal community and there is limited knowledge on how to compose a suitable species mixture. With the purpose of providing a highly productive, sustainable mixture for forage pastures in the Mixed Grass Prairie ecoregion, seven perennial species native to the region were selected. They are: nodding brome, blue bunch wheatgrass, western wheatgrass, side oats grama, little blue stem, purple prairie clover, and white prairie clover.

The objectives of this thesis are 1) to assess the productivity (forage yield), crude protein content of the forage swards to determine if species show complementarity, and if the forage swards affected the soil nitrogen level, with forage swards that include legumes increasing available nitrogen; 2) to determine how water limitation impacted above-ground productivity, and if it altered the predictive ability of the community characteristics resulting in a different optimum community required under drought stress; and 3) to assess more complex mixtures by developing and applying a method (using community characteristics to predict productivity) to rapidly and systematically screen potential communities for high productivity. Thus this research will determine if complex mixtures of these species could provide a sustainable, drought tolerant, non-invasive, productive rangeland for pasture use in the Mixed Grass Prairie ecoregion. This research also provides a systematic method to select the best mixtures which may aid in

screening species and community compositions, leading to mixtures that are more specifically designed to be stable, and highly productive for the region.

## 1.5 References


**Abouguendia, Z. 1990**. Range Plan Development - A practical guide to planning for managment and improvement of Saskatchewan rangeland. New Pasture and Grazing Technologies Project. Regina, SK. 52 pp.

**Aerts, R., Boot, R. G. A. and van der Aart, P. J. M. 1991**. The relation between above- and belowground biomass allocation patterns and competitive ability. Oecologia. **87**: 551-559.

**Balvanera, P., Pfisterer, A. B., Buchmann, N., He, J. S., Nakashizuka, T., Raffaelli, D. and Schmid, B. 2006**. Quantifying the evidence for biodiversity effects on ecosystem functioning and services. Ecol. Lett. **9**: 1146-1156.

**Bement, R. E., Barmington, R. D., Everson, A. C., Hylton, L. O., Jr. and Remmenga, E. E. 1965**. Seeding of abandoned croplands in the Central Great Plains. J. Range Manag. **18**: 53-59.

**Bertness, M. D. and Callaway, R. 1994**. Positive interactions in communities. Trends Ecol. Evol. **9**: 191-193.

**Brooker, R. W., Maestre, F. T., Callaway, R. M., Lortie, C. L., Cavieres, L. A., Kunstler, G., Liancourt, P., Tielbörger, K., Travis, J. M. J., Anthelme, F., Armas, C., Coll, L., Corcket, E., Delzon, S., Forey, E., Kikvidze, Z., Olofsson, J., Pugnaire, F., Quiroz, C. L., Saccone, P., Schiffers, K., Seifan, M., Touzard, B. and Michalet, R. 2008**. Facilitation in plant communities: the past, the present, and the future. J. Ecol. **96**: 18-34.

**Bruno, J. F., Stachowicz, J. J. and Bertness, M. D. 2003**. Inclusion of facilitation into ecological theory. Trends Ecol. Evol. **18**: 119-125.

**Bruynooghe, J. and Macdonald, R. 2008**. Managing Saskatchewan rangeland (revised edition). Saskatchewan Forage Council, Ducks Unlimited Canada, Agriculture and Agri-Food Canada - Prairie Farm Rehabilitation Administration, Saskatchewan Ministry of Agriculture, Saskatchewan Watershed Authority and the University of Saskatchewan.

**Buxton, D. R. 1996**. Quality-related characteristics of forages as influenced by plant environment and agronomic factors. Anim. Feed Sci. Technol. **59**: 37-49.

**Cahill, J. F. 2003**. Lack of relationship between below-ground competition and allocation to roots in 10 grassland species. J. Ecol. **91**: 532-540.

**Callaway, R. M. 1995**. Positive interactions among plants. Bot. Rev. **61**: 306-349.

**Callaway, R. M. 2007**. Positive interactions and interdependence in plant communities. Springer. Pp 415.

**Choler, P., Michalet, R. and Callaway, R. M. 2001**. Facilitation and competition on gradients in alpine plant communities. Ecology. **82**: 3295-3308.

**Cook, C. W., Stoddart, L. A. and Harris, L. E. 1956**. Comparative nutritive value and palatability of some introduced and native forage plants for spring and summer grazing. Utah Agr. Exp. Sta. Bull. **385**: Pp 39.

**Cooke, T. G. 1972**. Comparative nutritive values of forbs, grasses and shrubs. Pages 303-310 i*n* C.M. McKell, J.P. Blaisdell and J.R. Goodin (eds.). Wildland shrubs: Their biology and utilization. USDA forest service GTR INT-1.

**Craine, J. M., Tilman, D., Wedin, D., Reich, P., Tjoelker, M. and Knops, J. 2002**. Functional traits, productivity and effects on nitrogen cycling of 33 grassland species. Funct. Ecol. **16**: 563-574.

**Daubenmire, R. F. 1940**. Plant succession due to overgrazing in the *Agropyron* bunchgrass prairie of southeastern Washington. Ecology. **21**: 55-64.

**Díaz, S. and Cabido, M. 2001**. Vive la différence: plant functional diversity matters to ecosystem processes. Trends Ecol. Evol. **16**: 646-655.

**Doak, D. F., Bigger, D., Harding, E. K., Marvier, M. A., O'Malley, R. E. and Thomson, D. 1998**. The statistical inevitability of stability diversity relationships in community ecology. The American Naturalist. **151**: 264-276.

**Dorner, J. 2002**. An introduction to using native plants in restoration projects. http://www.nps.gov/plants/restore/pubs/intronatplant/intronatplant.pdf. Accessed 3 January 2013

**Frank, A. B. 1994**. Physiological comparisons of crested wheatgrass and western wheatgrass to water. J. Range Manag. **47**: 460-466.

**Ganskopp, D., Svejcar, T. and Vavra, M. 2004**. Livestock forage conditioning: bluebunch wheatgrass, Idaho fescue, and bottlebrush squirreltail. J. Range Manag. **57**: 384-392.

**Garnier, E., Navas, M. L., Austin, M. P., Lilley, J. M. and Gifford, R. M. 1997**. A problem for biodiversity-productivity studies: how to compare the productivity of multispecific plant mixtures to that of monocultures? Acta Oecol. **18**: 657-670.

**Gerrish, J. 2001.** Species stability in diverse pasture mixtures. Pages 212-216 *in* T. Terrill, ed. American Forage and Grassland Council, Vol 10, Proceedings. Amer Forage & Grassland Council, Georgetown.

**Gillen, R. L. and Berg, W. A. 2005**. Response of perennial cool-season grasses to clipping in the southern plains. Agron. J. **97**: 125-130.

**Goldberg, D. E. 1996**. Competitive ability: Definitions, contingency and correlated traits. Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences. **351**: 1377-1385.

**Goldberg, D. E., Grace, J. and Tilman, D. 1990**. Components of resource competition in plant communities. Perspectives on plant competition. Pp 27-49.

**Grime, J. P. 1974**. Vegetation classification by reference to strategies. Nature. **250**: 26-31.

**Grime, J. P. 1977**. Evidence for the existence of three primary strategies in plants and its relevance to ecological and evolutionary theory. The American Society of Naturalists. **111**: 1169-1194.

**Grime, J. P. and Hunt, R. 1975**. Relative growth-rate: Its range and adaptive significance in a local flora. J. Ecol. **63**: 393-422.

**Grman, E., Lau, J. A., Schoolmaster, D. R. and Gross, K. L. 2010**. Mechanisms contributing to stability in ecosystem function depend on the environmental context. Ecol. Lett. **13**: 1400-1410.

**Harper-Lore, B. 1996**. Using native plants as problem-solvers. Environ. Manag. **20**: 827-830.

**Harris, G. A. 1967**. Some competitive relationships between *Agropyron spicatum* and *Bromus tectorum*. Ecol. Monogr. **37**: 89-111.

**Heady, H. F. and Child, R. D. 1994**. Rangeland ecology and managment. Westview Press, Boulder, Colorado. Pp 524.

**Herbel, C. H., Ares, F. N. and Wright, R. A. 1972**. Drought effects on a semidesert grassland range. Ecology. **53**: 1084-1093.

**Hitchcock, A. S. 1971**. Manual of the grasses of the United States. Dover Publications.

**Hofmann, L., Ries, R. E., Karn, J. F. and Frank, A. B. 1993**. Comparison of seeded and native pastures grazed from mid-May through September. J. Range Manag. **46**: 251-254.

**Hogg, E. H. 1994**. Climate and the southern limit of the western Canadian boreal forest. Can. J. For. Res. **24**: 1835-1845.

**Holechek, J. L., Pieper, R. D. and Herbel, C. H. 2004**. Range animal nutrition. Pages 265-316 *in* Range management: Principles and practices. 5th Ed. Prentice-Hall, Inc. Englewood Cliffs, New Jersey.

**Hooper, D. U., Chapin, F. S., Ewel, J. J., Hector, A., Inchausti, P., Lavorel, S., Lawton, J. H., Lodge, D. M., Loreau, M., Naeem, S., Schmid, B., Setala, H., Symstad, A. J., Vandermeer, J. and Wardle, D. A. 2005**. Effects of biodiversity on ecosystem functioning: A consensus of current knowledge. Ecol. Monogr. **75**: 3-35.

**Jackson, R. D., Paine, L. K. and Woodis, J. E. 2010**. Persistence of native C4 grasses under high-intensity, short-duration summer bison grazing in the eastern tallgrass prairie. Restor. Ecol. **18**: 65-73.

**Jefferson, P. G., Iwaasa, A. D., Schellenberg, M. P. and McLeod, J. G. 2005**. Re-evaluation of native plant species for seeding and grazing by livestock on the semiarid prairie of western Canada. Prairie Forum. **30**: 85-106.

**Jefferson, P. G., McCaughey, W. P., May, K., Woosaree, J., MacFarlane, L. and Wright, S. M. 2002**. Performance of American native grass cultivars in the Canadian prairie provinces. Native Plants Journal. **3**: 24-33.

**Jefferson, P. G., McCaughey, W. P., May, K., Woosaree, J. and McFarlane, L. 2004**. Forage quality of seeded native grasses in the fall season on the Canadian prairie provinces. Can. J. Plant Sci. **84**: 503-509.

**Jin, L., Wang, Y., Iwaasa, A. D., Xu, Z., Schellenberg, M. P., Zhang, Y. G., Liu, X. L. and McAllister, T. A. 2012**. Effect of condensed tannins on ruminal degradability of purple prairie clover (*Dalea purpurea Vent.*) harvested at two growth stages. Anim. Feed Sci. Technol. **176**: 17-25.

**Johnson, D. A. and Asay, K. H. 1993**. Viewpoint: selection for improved drought response in cool-season grasses. J. Range Manag. **46**: 194-202.

**Karn, J. F., Ries, R. E. and Hofmann, L. 1999**. Season-long grazing of seeded cool-season pastures in the Northern Great Plains. J. Range Manag. **52**: 235-240.

**Kilcher, M. R. and Looman, J. 1983**. Comparative performance of some native and introduced grasses in southern Saskatchewan, Canada. J. Range Manag. **36**: 654-657.

**Kirwan, L., Luescher, A., Sebastia, M. T., Finn, J. A., Collins, R. P., Porqueddu, C., Helgadottir, A., Baadshaug, O. H., Brophy, C., Coran, C., Dalmannsdottir, S., Delgado, I., Elgersma, A., Fothergill, M., Frankow-Lindberg, B. E., Golinski, P., Grieu, P., Gustavsson, A. M., Hoglind, M., Huguenin-Elie, O., Iliadis, C., Jorgensen, M., Kadziuliene, Z., Karyotis, T., Lunnan, T., Malengier, M., Maltoni, S., Meyer, V., Nyfeler, D., Nykanen-Kurki, P., Parente, J., Smit, H. J., Thumm, U. and Connolly, J. 2007**. Evenness drives consistent diversity effects in intensive grassland systems across 28 European sites. J. Ecol. **95**: 530-539.

**Knapp, E. E. and Rice, K. J. 1997**. Ecotypes of native species: how local is local in restoration plantings. California Exotic Pest Plant Council. 1997 Symposium Proceedings. 5 pp.

**Knowles, R. P. 1987**. Productivity of grass species in the dark brown soil zone of Saskatchewan. Can. J. Plant Sci. **67**: 719-725.

**Lamb, E., Kennedy, N. and Siciliano, S. 2011**. Effects of plant species richness and evenness on soil microbial community diversity and function. Plant Soil. **338**: 483-495.

**Lehman, Clarence L. and Tilman, D. 2000a**. Biodiversity, stability, and productivity in competitive communities. Am. Nat. **156**: 534-552.

**Lehman, C. L. and Tilman, D. 2000b**. Biodiversity, stability, and productivity in competitive communities. Am. Nat. **156**: 534-552.

**Lhomme, J. p. and Winkel, T. 2002**. Diversity stability relationships in community ecology: Re-examination of the portfolio effect. Theor. Popul. Biol. **62**: 271-279.

**Loreau, M. and de Mazancourt, C. 2013**. Biodiversity and ecosystem stability: a synthesis of underlying mechanisms. http://dx.doi.org/10.1111/ele.12073.

**Maestre, F. T., Valladares, F. and Reynolds, J. F. 2005**. Is the change of plant–plant interactions with abiotic stress predictable? A meta-analysis of field results in arid environments. J. Ecol. **93**: 748-757.

**Mason, J. L. and Miltimore, J. E. 1959**. Increase in yield and protein content of native bluebunch and wheatgrass from nitrogen fertilization. Can. J. Plant Sci. **39**: 501-504.

**May, K., Stout, D. G., Willms, W. D., Mir, Z., Coulman, B., Fairey, N. A. and Hall, J. W. 1998**. Growth and forage quality of three *Bromus* species native to western Canada. Can. J. Plant Sci.: 597–603.

**McGraw, R. L., Shockley, F. W., Thompson, J. F. and Roberts, C. A. 2004**. Native legume species. Native Plants Journal. **5**: 152-159.

**Miller, R. F., Seufert, J. M. and Haferkamp, M. R. 1986**. The ecology and management of bluebunch wheatgrass (*Agropyron spicatum*): a review.

**Miller, T. E. 1994**. Direct and indirect species interactions in an early old-field plant community. The American Society of Naturalists. **143**: 1007-1025.

**Molano-Flores, B., Coons, J. M. and Cunningham, J. B. 2011**. Germination of two seed types from three *Dalea* species. Castanea. **76**: 266-271.

**Moncada, K. M., Ehlke, N. J., Muehlbauer, G. J., Sheaffer, C. C., Wyse, D. L. and DeHaan, L. R. 2007**. Genetic variation in three native plant species across the State of Minnesota. Crop Sci. **47**: 2379-2389.

**Monsen, S. B., Stevens, R. and Shaw, N. 2004**. Chapter 18. Grasses. Pages 295-424 *in* Restoring western ranges and wildlands, vol. 2. USDA Forest Service Gen. Tech. Rep. RMRS-GTR-136.

**Morgan, J. A., Skinner, R. H. and Hanson, J. D. 2001**. Nitrogen and $CO_2$ affect regrowth and biomass partitioning differently in forages of three functional groups. Crop Sci. **41**: 78-86.

**Muir, J. P., Pitman, W. D. and Foster, J. L. 2011**. Sustainable, low-input, warm-season, grass-legume grassland mixtures: mission (nearly) impossible? Grass Forage Sci. **66**: 301-315.

**Nicholson, R. A. and Bonham, C. D. 1977**. Grama (*Bouteloua Lag*.) communities in a southeastern Arizona grassland. J. Range Manag. **30**: 427-433.

**Oelmann, Y., Wilcke, W., Temperton, V. M., Buchmann, N., Roscher, C., Schumacher, J., Schulze, E. D. and Weisser, W. W. 2007**. Soil and plant nitrogen pools as related to plant diversity in an experimental grassland. Soil Sci. Soc. Am. J. **71**: 720-729.

**Padbury, G., Acton, D. F. and Stushnoff, C. T. 1998**. Ecoregions of Saskatchewan. Regina: Canadian Plains Research Center.

**Peek, J. M. 2010**. Pattern of herbivory, nitrogen content, and biomass of bluebunch wheatgrass on a mountain sheep habitat in central Idaho. Northwest. Sci. **84**: 386-393.

**Petersen, S. L., Roundy, B. A. and Bryant, R. M. 2004**. Revegetation methods for high-elevation roadsides at Bryce Canyon National Park, Utah. Restor. Ecol. **12**: 248-257.

**Phan, A. T. and Smith, S. R. 2000**. Seed yield variation in blue grama and little bluestem plant collections in southern Manitoba, Canada. Crop Sci. **40**: 555-561.

**Powell, M. C., Muntifering, R. B., Lin, J. C. and Chappelka, A. H. 2003**. Yield and nutritive quality of sericea lespedeza (*Lespedeza cuneata*) and little bluestem (*Schizachyrium scoparium*) exposed to ground-level ozone. Environ. Pollut. **122**: 313-322.

**Prairie Conservation Action Plan Partnership. 2005**. Saskatchewan prairie conservation action plan 2003-2008. Pp 46.

**Reich, P. B., Tilman, D., Naeem, S., Ellsworth, D. S., Knops, J., Craine, J., Wedin, D. and Trost, J. 2004**. Species and functional group diversity independently influence biomass accumulation and its response to CO2 and N. Proc. Natl. Acad. Sci. U. S. A. **101**: 10101-10106.

**Richards, R. T., Chambers, J. C. and Ross, C. 1998**. Use of native plants on federal lands: Policy and practice. J. Range Manag. **51**: 625-632.

**Robins, J. G., Jensen, K. B., Peel, M. D. and Waldron, B. L. 2009**. Establishment of warm-season grasses in summer and damage in winter under supplementary irrigation in a semi-arid environment at high elevation in western United States of America. Grass Forage Sci. **64**: 42-48.

**Roscher, C., Weigelt, A., Proulx, R., Marquard, E., Schumacher, J., Weisser, W. W. and Schmid, B. 2011**. Identifying population- and community-level mechanisms of diversity-stability relationships in experimental grasslands. J. Ecol. **99**: 1460-1469.

**Saskatchewan Forage Council. 2010**. The value of Saskatchewan's forage industry: A multi-level analysis.

**Schellenberg, M. P. 2008**. Biomass yield differences for introduced versus native grasses in mono- and poly- cultures in southwestern Saskatchewan. Abstract in: "Building Bridges: Grasslands to Rangelands". SRM-AFGC AGM 25-31 Louisville, KY. CD. Paper No. 2138.

**Schellenberg, M. P. and Banerjee, M. R. 2002**. The potential of legume-shrub mixtures for optimum forage production in southwestern Saskatchewan: A greenhouse study. Can. J. of Plant Sci. **82**: 357-363.

**Schellenberg, M. P., Biligetu, B. and Iwaasa, A. D. 2012**. Species dynamic, forage yield, and nutritive value of seeded native plant mixtures following grazing. Can. J. of Plant Sci. **92**: 699-706.

**Schellenberg, M. P., Holt, N. W. and Waddington, J. 1999**. Effects of grazing dates on forage and beef production of mixed prairie rangeland. Can. J. Anim. Sci. **79**: 335-341.

**Sheehan, C., Kirwan, L., Connolly, J. and Bolger, T. 2006**. The effects of earthworm functional group diversity on nitrogen dynamics in soils. Soil Biology and Biochemistry. **38**: 2629-2636.

**Simmers, S. M. and Galatowitsch, S. M. 2010**. Factors affecting revegetation of oil field access roads in semiarid grassland. Restor. Ecol. **18**: 27-39.

**Stubbendieck, J., Hatch, S. and Landholt, L. M. 2011**. North American Wildland Plants: A Field Guide. University of Nebraska Press.

**Symstad, A. J., Chapin, F. S., Wall, D. H., Gross, K. L., Huenneke, L. F., Mittelbach, G. G., Peters, D. P. C. and Tilman, D. 2003**. Long-term and large-scale perspectives on the relationship between biodiversity and ecosystem functioning. Bioscience. **53**: 89-98.

**Tannas, K. 2004**. Common plants of the western rangelands: Forbs. Vol 3. Alberta Agriculture, Food and Rural Development. Pp 505.

**Temperton, V. M., Mwangi, P. N., Scherer-Lorenzen, M., Schmid, B. and Buchmann, N. 2007**. Positive interactions between nitrogen-fixing legumes and four different neighbouring species in a biodiversity experiment. Oecologia. **151**: 190-205.

**Thorne, M. and Cardina, J. 2011.** Prairie grass establishment on calcareous reclaimed mine soil. J. Environ. Qual. **40**: 1824-1834.

**Tilman, D. 1996**. Biodiversity: Population versus ecosystem stability. Ecology. **77**: 350-363.

**Tilman, D. 1997**. Community invasibility, recruitment limitation, and grassland biodiversity. Ecology. **78**: 81-92.

**Tilman, D. 1999**. The ecological consequences of changes in biodiversity: A search for general principles. Ecology. **80**: 1455-1474.

**Tilman, D. and Elhaddi, A. 1992**. Drought and biodiversity in grasslands. Oecologia. **89**: 257-264.

**Tilman, D., Reich, P. B., Knops, J., Wedin, D., Mielke, T. and Lehman, C. 2001**. Diversity and productivity in a long-term grassland experiment. Science. **294**: 843-845.

**Tracy, B. F., Maughan, M., Post, N. and Faulkner, D. B. 2010**. Integrating Annual and Perennial Warm-season Grasses in a Temperate Grazing System. Crop Sci. **50**: 2171-2177.

**Tracy, B. F. and Sanderson, M. A. 2004**. Forage productivity, species evenness and weed invasion in pasture communities. Agric. Ecosyst. Environ. **102**: 175-183.

**Trenbath, B. R. 1974a**. Biomass productivity of mixtures. Adv. Agron. **26**: 177-210.

**Trenbath, B. R. 1974b**. Neighbor effects in Genus *Avena*. Comparison of weed species. J. Appl. Ecol. **11**: 111-125.

**United States Department of Agriculture National Resources Conservation Service Plant Materials Program. 2002a**. Purple Prairie Clover *Dalea purpurea* Vent. http://plants.usda.gov/java/profile?symbol=DAPU5. 11 January 2013.

**United States Department of Agriculture National Resources Conservation Service Plant Materials Program. 2002b**. Western Wheatgrass *Pascopyrum smithii* (Rydb.) Scribn. http://plants.usda.gov/java/profile?symbol=PASM. 31 July 2012. USDA Forest Service Gen. Tech. Rep. RMRS-GTR-136.

**Van Soest, P. J. 1982**. Nutritional ecology of the ruminant. O&B Books, Inc., Corvallis, OR. Pp 374.

**Volin, J. C., Reich, P. B. and Givnish, T. J. 1998**. Elevated carbon dioxide ameliorates the effects of ozone on photosynthesis and growth: Species respond similarly regardless of photosynthetic pathway or plant functional group. New Phytol. **138**: 315-325.

**Walker, B. 1995**. Conserving Biological Diversity through Ecosystem Resilience
La conservación de la diversidad biológica a través de la resiliencia de los ecosistemas. Conserv. Biol. **9**: 747-752.

**Walker, B., Kinzig, A. and Langridge, J. 1999**. Original articles: Plant attribute diversity, resilience, and ecosystem function: The nature and significance of dominant and minor species. Ecosystems. **2**: 95-113.

**Wang, P., Stieglitz, T., Zhou, D. W. and Cahill Jr, J. F. 2010**. Are competitive effect and response two sides of the same coin, or fundamentally different? Funct. Ecol. **24**: 196-207.

**Weaver, J. E. 1919**. The ecological relations of roots. Carnegie Inst. Wash. Pub. **286**: Pp 128.

**Weaver, J. E. 1958**. Summary and interpretation of underground development in natural grassland communities. Ecol. Monogr. **28**: 55-78.

**Weigelt, A., Steinlein, T. and Beyschlag, W. 2005**. Competition among three dune species: the impact of water availability on below-ground processes. Plant Ecol. **176**: 57-68.

**Weigelt, A., Weisser, W. W., Buchmann, N. and Scherer-Lorenzen, M. 2009**. Biodiversity for multifunctional grasslands: equal productivity in high-diversity low-input and low-diversity high-input systems. Biogeosciences. **6**: 1695-1706.

**Whitbread, A. M., Hall, C. A. and Pengelly, B. C. 2009**. A novel approach to planting grass-legume pastures in the mixed farming zone of southern inland Queensland, Australia. Crop Pasture Sci. **60**: 1147-1155.

**White, L. M. 1986**. Forage yield and quality of warm- and cool-season grasses. J. Range Manag. **39**: 264-268.

**Wight, J. R. and White, L. M. 1974**. Interseeding and pitting on a sandy range site in eastern Montana. J. Range. Manage. **27**: 206-210.

**Wilson, A. M., Harris, G. A. and Gates, D. H. 1966**. Cumulative effects of clipping on yield of bluebunch wheatgrass. J. Range. Manage. **19**: 90-91.

**Wilson, S. D. and Gerry, A. K. 1995**. Strategies for mixed-grass prairie restoration: Herbicide, tilling, and nitrogen manipulation. Restor. Ecol. **3**: 290-298.

**Yachi, S. and Loreau, M. 1999**. Biodiversity and ecosystem productivity in a fluctuating environment: The insurance hypothesis. Proc. Natl. Acad. Sci. USA. **96**: 1463-1468.

## Chapter 2 Preamble

This chapter evaluates the early establishment productivity, crude protein concentration , and effect on soil nitrogen of forage swards of the seven native perennial species in monoculture, biculture, and an all-species mixture. These forage swards were planted in two sites, Saskatoon and Swift Current, Saskatchewan. Productivity was strongly related to western wheatgrass in the community, legumes had the highest crude protein concentration, and these results were consistent between sites. Establishment was different between species and between sites. Western wheatgrass was important to achieve high yields, but yields were not reduced when it was planted in mixtures even though the seeding density of western wheatgrass was reduced. Therefore, including other species in the forage swards did not result in a penalty for yield as long as western wheatgrass was included. Thus, other species should be retained as they may benefit the community more in other environmental conditions, or as the stands mature over years. Soil nitrogen was reduced with increased yield but effects of legumes are not yet evident.

This chapter relates to the overall thesis by addressing study objective 1) to assess the productivity (forage yield), crude protein content of the forage swards to determine if species show complementarity, and if the forage swards affected the soil nitrogen level, with forage swards that include legumes increasing available nitrogen.

# 2 EARLY PRODUCTIVITY AND CRUDE PROTEIN CONTENT OF ESTABLISHING FORAGE SWARDS COMPOSED OF COMBINATIONS OF NATIVE GRASS AND LEGUME SPECIES IN MIXED-GRASSLAND ECOREGIONS

## 2.1 Abstract

We evaluated the early establishment productivity of forage swards of native, perennial, cool and warm season grasses, and legumes as they have the potential to provide non-invasive, productive, and drought resistant rangelands. Seven species with high agronomic potential and a broad native geographic distribution were selected for testing including: nodding brome [*Bromus anomalus* (Coult.)], blue bunch wheatgrass [*Pseudoregneria spicata* (Pursh)], western wheatgrass [*Pascopyrum smithii* (Rydb.)], side oats grama [*Bouteloua curtipendula* (Michx.)], little blue stem [*Schizachyrium scoparium* (Michx.)], purple prairie clover [*Dalea purpurea* (Vent.)], and white prairie clover [*Dalea candida* (Willd.)]. Forage swards, including all seven monocultures, 21 two-species mixtures and a mixture with all species, were planted in two sites, Saskatoon and Swift Current, Saskatchewan. Western wheatgrass (WWG) had the highest overall plant density and the strongest effect on the forage yield of the forage swards; however, productivity and crude protein content were not reduced when other species were also included in the forage sward. *Dalea* spp. did not establish as well as the other species, but had the highest crude protein concentrations. This work provides insight in forage sward development at the establishment stage; additional work is required to determine long-term species impacts for well established forage swards.

## 2.2 Introduction

There is a growing interest in the use of native perennial species for seeded rangeland and reclamation following disturbance as native species may increase carbon sequestration, improve wildlife habitat, lower agronomic inputs, and extend the grazing season (Richards et al. 1998; Symstad et al. 2003; Jefferson et al. 2004; Jefferson et al. 2005; Prairie Conservation Action Plan Partnership 2005; Weigelt et al. 2009; Simmers and Galatowitsch 2010; Muir et al. 2011; Schellenberg et al. 2012). Diverse forage swards composed of native species have the potential to be as productive as tame monocultures in a greater range of environmental conditions (Schellenberg 2008). Ideally, a forage sward would be nutritious, and provide forage for the entire spring and summer.

In the native Mixed Grass Prairie ecoregion, the major functional plant groups include $C_3$ and $C_4$ grasses, and forbs. Here we define functional groups in terms of the ecological significance as $C_3$ grasses, $C_4$ grasses, and legumes, although other classification systems have been suggested (see Díaz and Cabido 2001). Typically, grass species provide most of the digestible energy with $C_3$ grasses, the dominant group, producing the bulk of the forage yield in these areas (Schellenberg and Banerjee 2002; Muir et al. 2011). $C_3$ grasses grow early in the cool season and are dormant later in the growing season, whereas $C_4$ grasses initiate growing later in the season but continue growing through the summer which can help to maintain forage yield and thus animal yield by providing new nutritious biomass later in the season (Cooke 1972; Lehman and Tilman 2000; Tilman et al. 2001; Schellenberg et al. 2012).

Including $C_4$ and legume species is an important means to increase available crude protein in a forage sward. $C_3$ grasses provide high nutritional quality early in the growing season,

and $C_4$ grasses and legumes provide high nutritional quality later in the growing season (Buxton 1996; Holechek et al. 2004). This is due to crude protein concentrations being highest in young plant tissue and declining as plants mature (Muir et al. 2011). Additionally, functional groups differ in their concentration of crude protein and the rate of decline throughout the season, for example, legumes are noted for higher concentrations of crude protein but do not cure as well as grasses (Cooke 1972; Schellenberg and Banerjee 2002; McGraw et al. 2004). Therefore, a mixture of $C_3$'s, $C_4$'s, and legumes may supply higher sequential crude protein concentrations over the growing season. Legumes also fix atmospheric nitrogen into the soil and if released through mineralization can facilitate growth of grasses and reduce fertilizer requirements in nitrogen deficient rangeland systems (Callaway 1995; Brooker et al. 2008; Whitbread et al. 2009; Muir et al. 2011). Adequate soil nitrate can increase forage yield as nitrogen uptake of non-legume species is increased (Oelmann et al. 2007; Temperton et al. 2007).

Combinations of species or functional groups may show additivity or complementarity which can increase productivity (Hooper et al. 2005; Brooker et al. 2008). In this study, we assessed the suitability of forage swards in the early establishment phase of the communities, composed of mixtures of native $C_3$ and $C_4$ grasses, and legumes, for use as seeded rangeland. The objectives of this study were to: 1) Assess the productivity (forage yield) of the forage swards to determine if species show complementarity; 2) Assess the crude protein content of the forage swards; and 3) Assess if the forage swards affected the soil nitrogen level, with forage swards that include legumes increasing available nitrogen

## 2.3 Materials and methods

### *2.3.1 Site*

Field experiments were conducted at two sites in Saskatchewan: Agriculture and Agri-Food Canada (AAFC) Saskatoon Research Center, Saskatoon (52°11′N, 106°32′W), and AAFC Semiarid Prairie Agricultural Research Center (SPARC), Swift Current (50°25′N, 107°44′W). Saskatoon is located in the moist mixed grass ecoregion and has Dark Brown soil (clay/clay-loam) (Coulman pers. comm.; Acton and Ellis 1978), while Swift Current is located in the dry mixed grass ecoregion and has Brown soil (Swinton loam) (Schellenberg pers. comm; Ayers et al. 1985) described in Table 2.1. For the Swift Current site, all weather data was collected from AAFC SPARC. For the Saskatoon site, weather data was collected for 2010 and 2011 from the AAFC Saskatoon Research Centre, and the 1971-2000 climate normals from Environment Canada, Saskatoon, Saskatchewan Research Council Station (Fig. 2.1). Generally, precipitation was higher than normals for 2010 in Saskatoon, and for both years in Swift Current. Analysis of forage sward impact on soil moisture is included in the appendix.

**Table 2.1.** Soil properties of the Saskatoon and Swift Current study sites.

| Characteristics | Saskatoon | Swift Current |
|---|---|---|
| pH | 6.6 | 7.4 |
| $NO_3$-N (μg g–1) | 1.8 | 15.0 |
| Extractable P (μg g–1) | 5.8 | 7.6 |
| Extractable K (μg g–1) | 457.5 | 300.0 |
| Extractable S (μg g–1) | 3.7 | 2.6 |

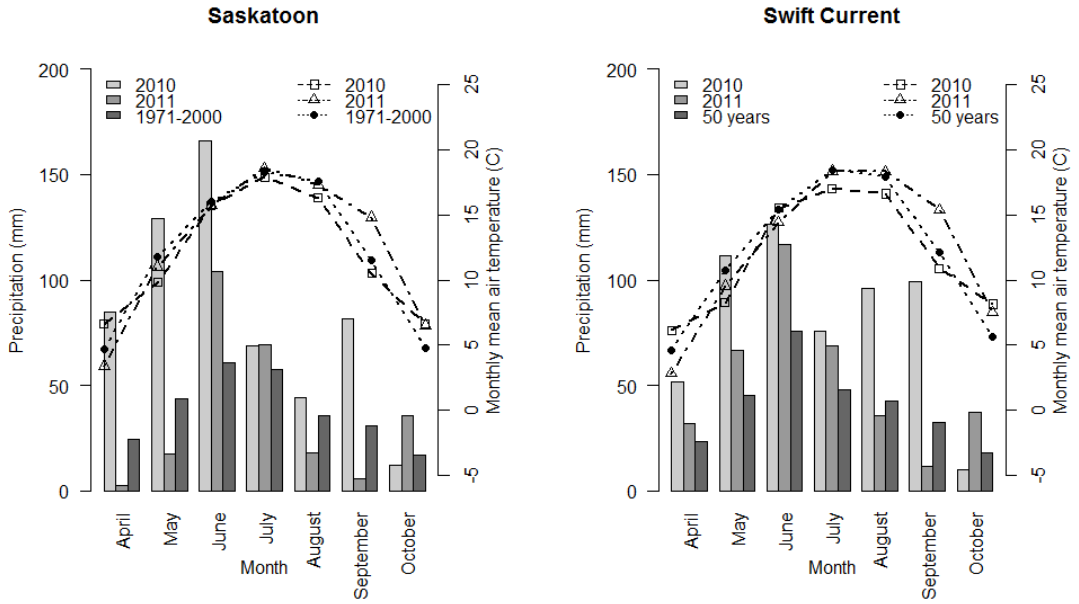


**Figure 2.1.** Monthly mean precipitation (bars) and air temperature (lines) during 2010, 2011, and the long term average.

### *2.3.2 Species*

Seven native, perennial species which have, and are continuing to be evaluated in various breeding programs located in Saskatoon, Swift Current, and Lethbridge, were selected for testing. These species have high agronomic potential (reasonable plant size, plant nutritional quality, cost, availability, and the ability to work with conventional machinery), a broad native geographic distribution, and include $C_3$ grasses: nodding brome (*Bromus anomalus* (Coult.), B), blue bunch wheatgrass (*Pseudoregneria spicata* (Pursh), BWG), western wheatgrass (*Pascopyrum smithii* (Rydb.), WWG), $C_4$ grasses: side oats grama (*Bouteloua curtipendula* (Michx.), SOG), little blue stem (*Schizachyrium scoparium* (Michx.), LBS), and legumes: purple prairie clover (*Dalea purpurea* (Vent.), PPC), white prairie clover (*Dalea candida* (Willd.), WPC). All seed provided was from the aforementioned breeding programs 2009 crop year.

### *2.3.3 Experimental Design*

In Saskatoon, a split-plot block design was seeded with three replicates (due to area restrictions) of 29 treatments July 21st, 2010. In Swift Current, a split-plot block design was seeded with four replicates of 30 treatments in June 14th, 2010. Treatments or 'forage sward' included monocultures, every two-species combination (biculture), and an all species combination. In Swift Current, one additional treatment of an unseeded plot was included as a reference site for soil nutrient analysis. Legumes were scarified and treated with an F-culture inoculant (Smith et al. 1988) to reduce dormancy and promote bacterial symbioses. Prior to seeding, both sites had been summer fallowed in the previous year. The Saskatoon plots were 3.75x6 m with 12 rows spaced 30.5 cm apart and seeded with a disc air seeder at a seeding depth of 1.3 cm. Plots were hand hoed between rows in 2010 for weed control. Weed control in the Swift Current site was done prior to seeding where plots sprayed in the spring of 2010 with Roundup WeatherMAX® (Monsanto Canada Inc., Winnipeg, Canada) (0.82 L $ha^{-1}$) and eleven days later with 2-4DB Cobutox® 625 (Interprovincial Cooperative Limited, Winnipeg, Canada) (2.47 L $ha^{-1}$). Plots were 4x8 m and seeded with 12 rows spaced 22.5 cm apart and seeded with a press drill at a seeding depth of 1.3 cm. Grass and legume species in monocultures were seeded at a rate to achieve equivalent seed germination between species of 100 seeds/m and 200 seeds/m (pure live seed counts), respectively (Schellenberg et al. unpublished data). Biculture forage swards, and the all species forage sward were seeded at half, and one seventh of the monoculture forage swards seeding rate, respectively.

Plant density was measured to estimate seedling establishment in the fall of 2010 by counting seedlings along one randomly chosen meter of two central rows in Saskatoon and four central rows in Swift Current. For both sites, the number of plants per meter was averaged for

each of the plots. The plots were split in half to harvest the forage yield twice: in the Mid-Season (end of June), and in the Late-Season harvest (end of August). Forage yield was measured by clipping shoots of all plants of the seeded species at ground level within two subsamples of the half plots (0.25 $m^2$ quadrats) with the exception of the Saskatoon Late harvest, where forage yield from only one subsample (quadrat) was collected. The shoots were dried, weighed, analyzed for nitrogen content, and crude protein concentration of the shoots was calculated by multiplying total Kjeldahl N by 6.25 (Noel and Hableton 1976). Percent crude protein yield was calculated by multiplying the % crude protein by the forage yield in each forage sward.

In Swift Current only, soil nitrate was measured in a subset of the plots to assess for forage sward treatment effects. The subset included three repetitions of the monocultures, bicultures (except for those containing LBS or WPC as these treatments were not included arbitrarily to reduce the number of probes required), and the all species forage sward. Soil cores were collected from each plot in the fall of 2010 as a baseline for nitrate in the rooting zone (top six inches) and analysed for total soil nitrate content ($NO_3$-N) using the technique of Hamm et al. (1970) and an automated combustion technique (Carlo Erba™, Milan, Italy). In the 2011 growing season, nitrate ($NO_3$-N) availability in the soil was measured using Plant Root Simulator ($PRS^{TM}$) probes (Western Ag Innovations, Inc., Saskatoon, Canada, Qian and Schoenau 2002). Probes were placed in the center of each plot, and replaced monthly through the growing season. The cumulative available soil nitrate was used in analyses.

### *2.3.4 Statistical Methods*

#### *2.3.4.1 Mixed Models and Model Selection Approach*

Mixed effect models were used in all analyses to avoid pseudoreplication as treatments were aggregated into blocks, and subsampled within plots. For all analyses a model selection approach was used where non-significant model terms ($p>0.05$) were removed in a stepwise manner (Crawley 2007) to identify significant terms and interactions. In this approach, power increases as unimportant terms are removed, and when sample size is small, relationships may be revealed that were previously unclear. Once the model contained only significant terms and if a categorical fixed variable was significant, statistical differences were identified between forage swards by sequentially combining groups with the smallest difference in mean until a significant increase in Akaike's Information Criterion (AIC) occurred (Akaike 1974). As a rule of thumb, a significant increase in AIC is a change of greater than two and indicates a reduction in fit; however, data were explored beyond this two point change to ensure that the final model was stable and not merely a transitional increase before a much lower AIC in later models. The final models presented contain only significant terms and represent the minimally adequate (best) model with the lowest AIC. In each analysis, the fit of a model with a linear relationship is compared to the fit of a null model with only an intercept using the "nlme" function with method= "ML" in the R package (R Development Core Team 2011). Available soil nitrate data and plant density data was log transformed, and all forage yield data was $\log(x+1)$ transformed to meet the assumption of the tests.

#### *2.3.4.2 Soil*

The effect of the forage swards on available soil nitrate over the growing season in the Swift Current site was tested using a mixed model and the model selection approach described above, where the initial model included plant available soil nitrate as the response variable, 'baseline' nitrate (to account for any differences in the plots before seeding), forage sward, forage yield, and the forage sward by forage yield interaction as fixed effects, and block as a random effect. The models involving the interaction term could not be solved, likely because of unbalanced sampling due to missing or damaged probes; only the main effects were reported. As the best model contained only forage yield (a continuous variable), it is equivalent to a simple linear regression.

#### *2.3.4.3 Plant density*

The effect of forage sward and site on plant density was tested using a mixed model and the model selection approach described above, where the model included plant density as the response variable, the site, forage sward, and the forage sward by site interaction as fixed effects, and block as a random effect. As plant density differed significantly between sites, the stepwise model selection process was performed separately for each site using a mixed model and model selection approach where the model included plant density as the response variable, forage sward as a fixed effect and block as a random effect.

**Table 2.2.** F-statistics and p-values indicating statistical significance for the complex effects of plant density, site, harvest season, forage sward, and their interactions on the forage yield of native grass and legume forage swards in monocultures, two species mixtures, and a seven species mixture, in two sites: Saskatoon and Swift Current, Saskatchewan.

| Fixed effects | F- statistic | p-value |
|---|---|---|
| Plant density | $F_{1,47}$=354.29 | p<0.001 |
| Site | $F_{1,47}$=876.14 | p<0.001 |
| Harvest Season | $F_{1,47}$=115.96 | p<0.001 |
| Forage Sward | $F_{28,47}$=77.46 | p<0.001 |
| Plant density : Site | $F_{1,47}$=2.49; | p=0.121 |
| Plant density: Harvest Season | $F_{1,47}$=0.09 | p=0.768 |
| Site: Harvest Season | $F_{1,47}$=2.40 | p=0.128 |
| Plant density : Forage Sward | $F_{28,47}$=2.66 | p=0.002 |
| Site: Forage Sward | $F_{28,47}$=2.85 | p=0.001 |
| Harvest Season : Forage Sward | $F_{28,47}$=0.47 | p=0.982 |
| Plant density : Site: Harvest Season | $F_{1,47}$=10.28 | p=0.002 |
| Plant density : Site: Forage Sward | $F_{28,47}$=1.45 | p=0.127 |
| Plant density : Harvest Season : Forage Sward | $F_{28,47}$=1.79 | p=0.038 |
| Site: Harvest Season : Forage Sward | $F_{28,47}$=1.15 | p=0.330 |
| Plant density : Site : Harvest Season : Forage Sward | $F_{28,47}$=1.04 | p=0.439 |

The effect of forage sward on forage yield was tested using mixed models and the model selection approach described above, where the initial model included forage yield as the response variable, plant density (because of potentially differential establishment of the species), forage sward, and the plant density by forage sward interaction as fixed effects, and block and subsample as random effects in the Saskatoon Mid-Season and both Swift Current analyses. Only block was included (and not subsample) as a random effect in all of the models for

Saskatoon Late-Season because only one subsample was collected. The best models for both seasons in Saskatoon and the Swift Current Mid-Season contained only forage sward and forage yield as main effects, and the initial models were also the best models for the Swift Current Late-Season.

#### *2.3.4.4 Forage yield*

The effect of forage sward on forage yield across site and harvest season was tested using an a priori mixed model and the model selection approach described above, where the model included forage yield as the response variable, plant density, site, harvest season, forage sward, and all interactions as fixed effects, and block and plot as random effects. There were complex three way interactions between all model terms, so the data was analyzed separately between site and harvest season in all further analyses (Table 2.2).

#### *2.3.4.5 Crude Protein*

The effect of forage sward on crude protein concentrations was tested using mixed models and the model selection approach described above, where the model included percent crude protein as the response variable, forage sward as a fixed effect, and block as a random term. Data were unbalanced because of insufficient plant material for crude protein to be processed.

## 2.4 Results

### *2.4.1 Soil*

Available soil nitrate was not significantly explained by 'baseline' or forage sward, but declined significantly as forage yield increased (Table 2.3, Fig. 2.2). One outlying data point is evident in

Figure 2.2, however, the removal of this data point did not alter these result; outlier is included in data and results.

**Table 2.3.** F-statistics and p-values indicating statistical significance for the effect of 'baseline' nitrate, forage sward, and forage yield on available soil nitrate over the growing season in Swift Current, Saskatchewan.

| Fixed effects | F- statistic | p-value |
|---|---|---|
| 'Baseline' Nitrate | $F_{1, 43}$=1.06 | p=0.310 |
| Forage Sward | $F_{17, 26}$=1.23 | p=0.307 |
| Forage Yield | $F_{1, 43}$= 24.26 | p<0.001 |

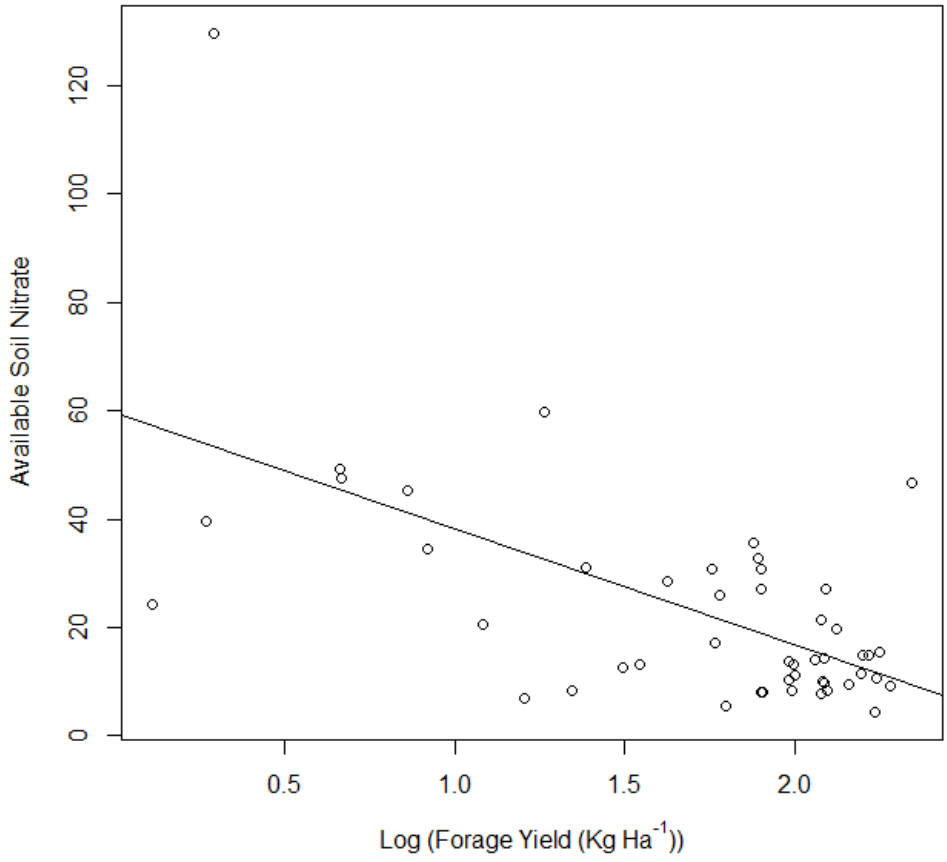


**Figure 2.2.** Regression of available soil nitrate (micro grams/10cm^2/growing season) versus log of the forage yield of all forage swards in Swift Current, Saskatchewan. Available soil nitrate decreased as forage yield increased (F1, 43= 24.26, p<0.001, R2 = 0.36, y = (-21.44) x + 59.56).

### *2.4.2 Plant Density*

Plant density differed between sites and forage swards, with a significant interaction (Table 2.4, Fig. 2.3). At both sites, legumes did not establish as well as the other species in the first year. Subsequent establishment was not collect, thus we can only infer long term establishment rates from the biomass data. WWG in monoculture and in combination with LBS and B had the highest plant density at both sites; however, differences between forage swards were less pronounced in Saskatoon than in Swift Current.

**Table 2.4.** F-statistics and p-values indicating statistical significance for the effect of site, forage sward, and their interaction on plant density of native grass and legume forage swards in monocultures, two species mixtures, and a seven species mixture, in two sites: Saskatoon and Swift Current Saskatchewan.

| Fixed effects | F- statistic | p-value |
|---|---|---|
| Site | $F_{1,142}$=47.62 | p<0.001 |
| Forage Sward | $F_{28,142}$=10.79 | p<0.001 |
| Site : Forage Sward | $F_{28,142}$=4.1324 | p<0.001 |

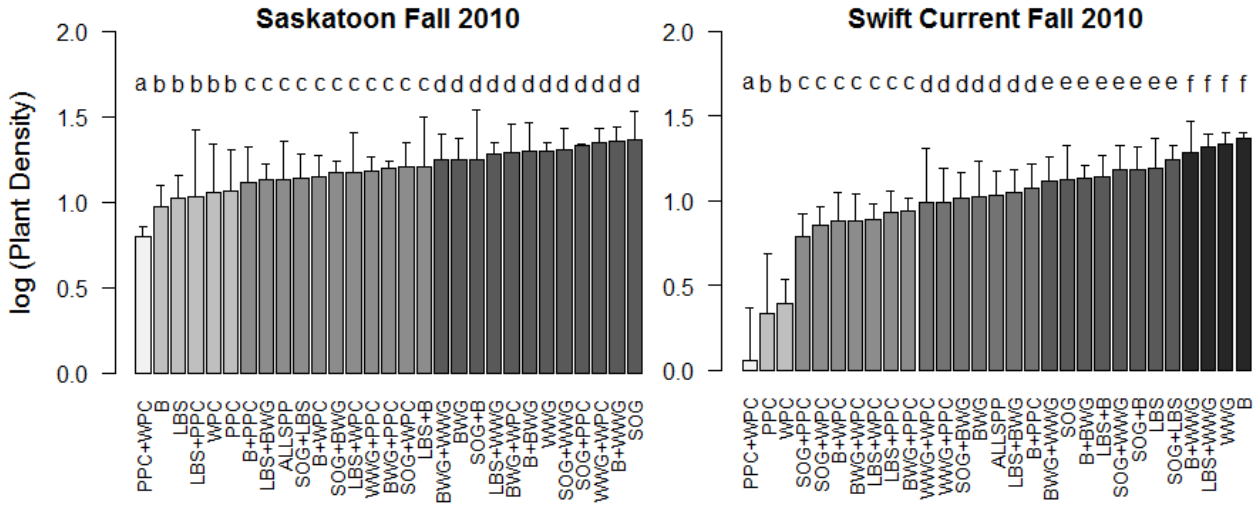


**Figure 2.3.** Log of plant density of each forage sward at each site, ordered according to rank. Means of four replications; where mean differences of forage swards were compared at the 95% level. Plant density differed significantly between forage swards, indicated by different letters. Error bars represent one standard deviation around the mean.

### *2.4.3 Forage Yield*

Forage yield differed between the forage swards, and increased with plant density (Table 2.5). In both sites, at both harvest times, monocultures and combinations of the legumes and $C_4$ grasses ranked low, while forage swards containing western wheatgrass ranked high (Fig. 2.4). Nodding brome and blue bunch wheatgrass monocultures (the other $C_3$ grasses) and in bicultures with the $C_4$ and legumes ranked mid-range. The all species forage sward ranked among the top 3 most productive groups in all cases. More specifically, in Saskatoon the wheatgrass forage sward (BWG+WWG) ranked highest in the Mid-Season, and all swards in the highest ranking group in the Late-Season contained WWG. In Swift Current, WWG monoculture and WWG with LBS, B, and PPC were ranked highest in the Mid-Season, and WWG with LBS, and B were ranked highest in the Late-Season.

**Table 2.5.** F-statistics and p-values indicating statistical significance for the effect of plant density, forage sward, and their interaction on forage yield (Kg/Ha) of native grass and legume forage swards in monocultures, two species mixtures, and a seven species mixture, in two sites: Saskatoon and Swift Current Saskatchewan. NS indicates a non-significant term that was removed from the model.

| Fixed Effects | Statistics | Saskatoon | | Swift Current | |
|---|---|---|---|---|---|
| | | Mid-Season | Late-Season | Mid-Season | Late-Season |
| Plant density | F-statistic | $F_{1,162}$=72.97 | $F_{1,78}$=69.77 | $F_{1,205}$=1112.31 | $F_{1,\ 203}$=582.19 |
| | p-value | p<0.001 | p<0.001 | p<0.001 | p<0.001 |
| Forage Sward | F-statistic | $F_{5,162}$=156.46 | $F_{5,78}$=133.76 | $F_{9,205}$=179.48 | $F_{10,\ 203}$=76.16 |
| | p-value | p<0.001 | p<0.001 | p<0.001 | p<0.001 |
| Plant density | F-statistic | NS | NS | $F_{9,\ 205}$=5.07 | $F_{10,\ 203}$=3.19 |
| : Forage Sward | p-value | NS | NS | p<0.001 | p=0.008 |

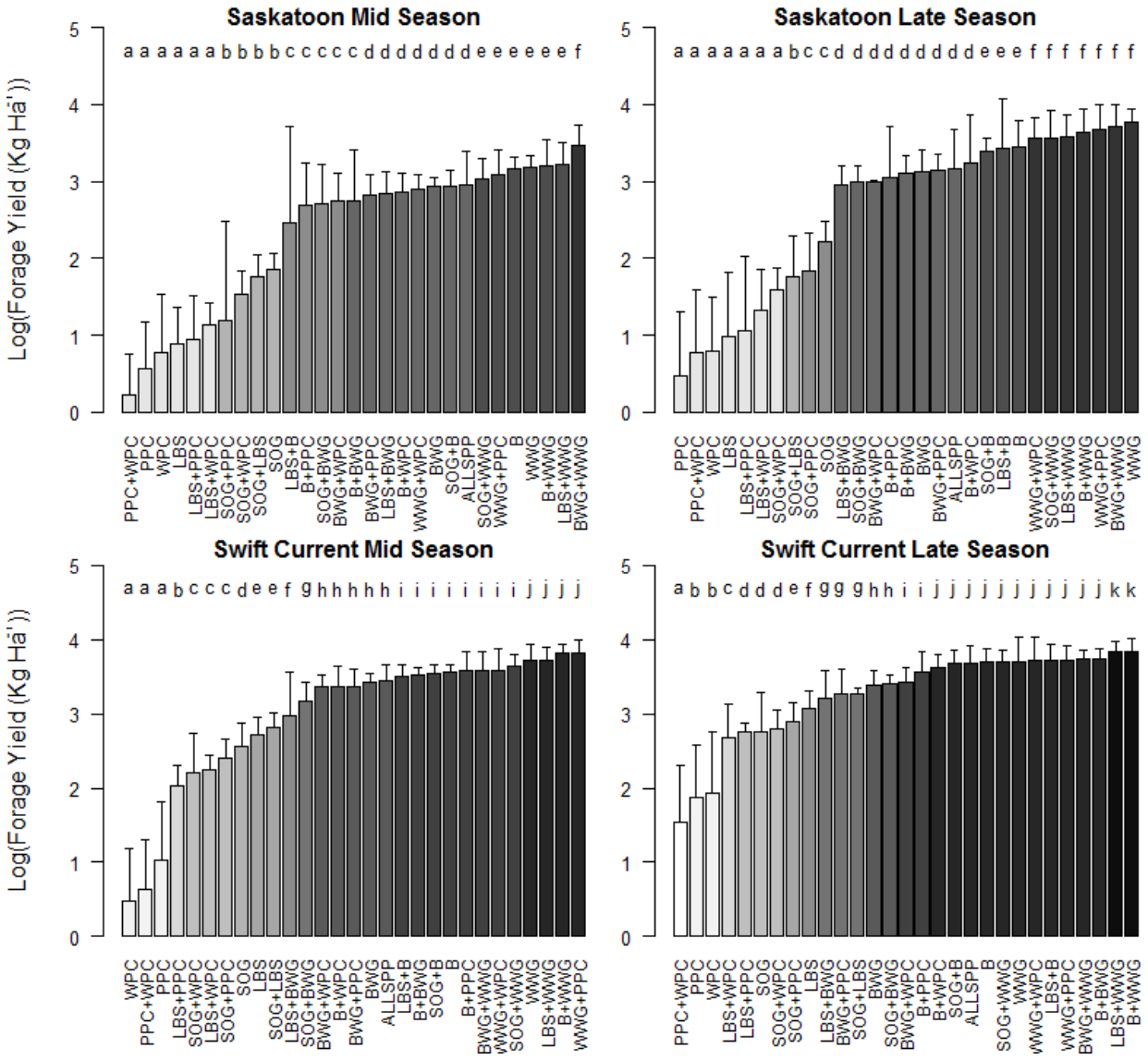


**Figure 2.4.** Log of forage yield (kg ha^-1) of forage swards at the Mid and Late Season, in Saskatoon and Swift Current, Saskatchewan, ordered according to rank. Means of four replications; where mean differences of forage swards were compared at the 95% level. Forage yield differed significantly between forage swards, indicated by different letters. Error bars represent one standard deviation around the mean.

### *2.4.4 Crude Protein*

Crude protein differed significantly between swards (Table 2.6). Percent crude protein for each treatment was highly variable across time of harvest and site, though the legume monocultures and PPC+WPC were consistently highly ranked (Fig. 2.5). In Saskatoon Mid-Season, the swards

with the highest crude protein include the LBS monoculture, WWG monoculture, WWG with the legumes and $C_4$ grasses, and the $C_4$ grasses with PPC. The highest ranking bicultures were LBS+PPC, SOG+WWG and WWG+PPC in the Mid-Season, and were also the highest ranking in the Late-Season, along with PPC+WPC.

**Table 2.6.** F-statistics and p-values indicating statistical significance for the effect of forage sward on the crude protein concentration of native grass and legume forage swards in monocultures, two species mixtures, and a seven species mixture, in two sites: Saskatoon and Swift Current Saskatchewan.

| Fixed Effect | Statistics | Saskatoon | | Swift Current | |
|---|---|---|---|---|---|
| | | Mid-Season | Late-Season | Mid-Season | Late-Season |
| Forage Sward | F-statistic | $F_{3,62}=72.36$; | $F_{5,60}=44.18$; | $F_{4,61}=55.66$; | $F_{3,107}=32.18$; |
| | p-value | $p<0.001$ | $p<0.001$ | $p<0.001$ | $p<0.001$ |

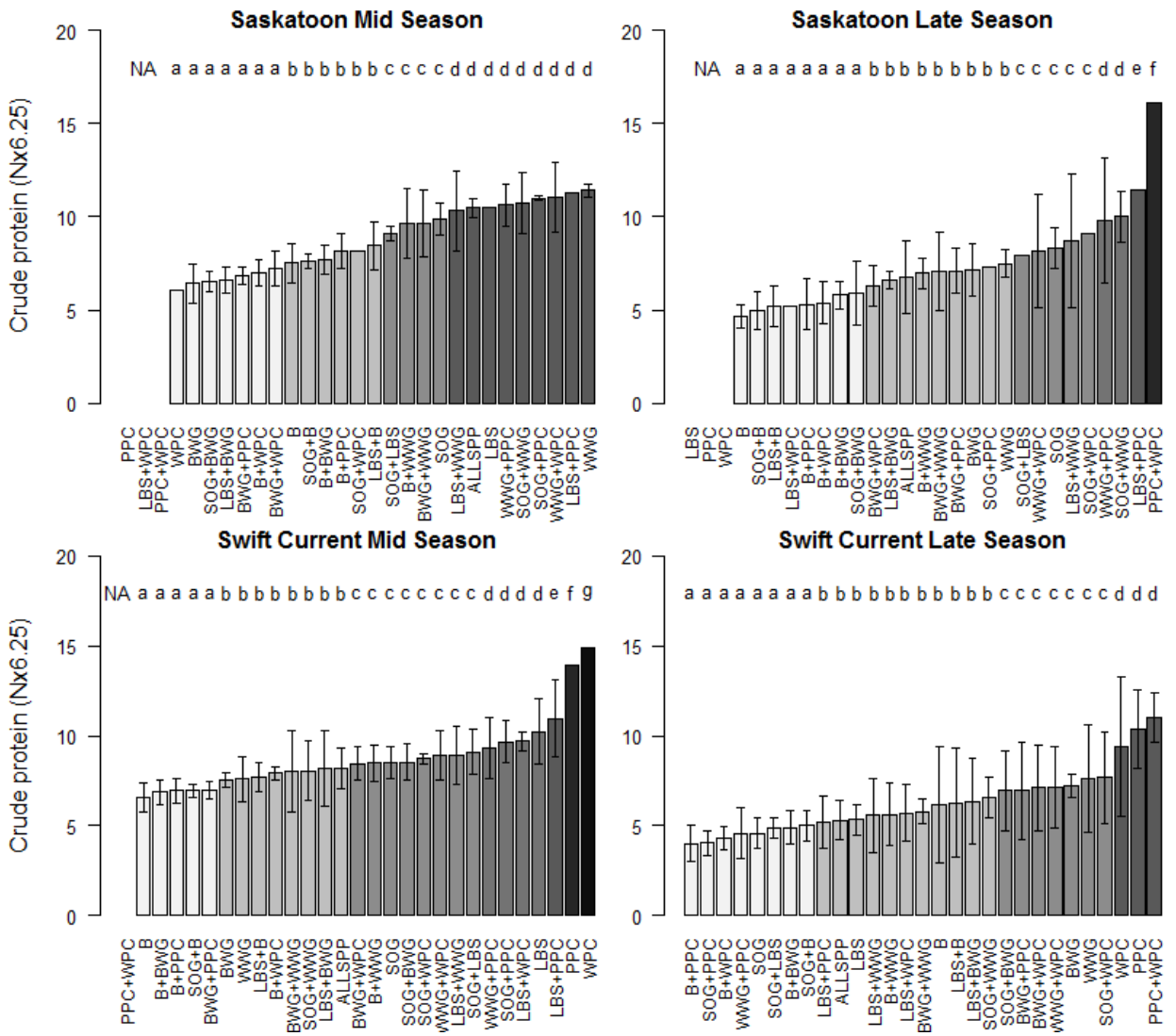


**Figure 2.5.** Crude protein (%) of each forage sward for each season harvest at each site, ordered according to rank. The forage swards that did not have enough shoot biomass to analyse for crude protein are represented as missing bars. Means of four replications; where mean differences of forage swards were compared at the 95% level. Crude protein concentration differed significantly between forage swards, indicated by different letters. Error bars represent one standard deviation around the mean, missing error bars indicate n=1.

## 2.5 Discussion

The most productive species was WWG, which performed better than all other monocultures (except B in the Saskatoon Mid Season). However, even when WWG is seeded at half of the seeding rate, the mixed forage sward was still as productive as WWG in monoculture. Therefore,

including $C_4$ grasses and legumes in addition to WWG, did not reduce the productivity of the forage sward. WWG is palatable, nutritious, digestible, cures well on the stem, and has low neutral detergent fiber (NDF) concentration suitable to maintain the dietary requirements of a 5 year old, non-lactating Angus beef cow in the second trimester of pregnancy (National Research Council 2000; Jefferson et al. 2004). WWG has extremely high potential as a perennial forage sward, as it is easily established and grows into a dense stand with sod-forming rhizomes (National Research Council 2000; United States Department of Agriculture National Resources Conservation Service Plant Materials Program 2002). As the only sod-forming $C_3$ in this study it may represent a more competitive life strategy, possibly due to high early relative growth rate (Chapter 4) and colonization potential (Monsen et al. 2004). This likely contributed to it being the most productive species in the early establishment of these forage swards, especially as drought conditions did not limit WWG (Wang and Schellenberg 2012). Additionally, as a native species it does not incur the associated negative invasiveness of other highly productive sod-forming introduced species such as smooth brome (Bahm et al. 2011). Wheatgrasses have been shown to be successful initially after disturbance where available nitrogen is higher (Hammermeister et al. 2003) and WWG is a competitive dominant as both a seedling and more obviously as an adult (Zhang and Lamb 2011). However WWG may not maintain productivity as available nitrogen becomes depleted (Hammermeister et al. 2003) or under grazing pressure (Branson 1953). Forage species can be classified by their response to grazing as increasers, decreasers, or invaders (Dyksterhuis 1949). WWG is a decreaser in Mixed Grassland grazing systems, thus monocultures of WWG may result in reduced long-term forage yield (Branson 1953; Holechek et al. 2004). Including increaser species such as little bluestem in the forage sward is thus likely important for maintaining forage yield. Including other species in

combination with WWG did not reduce forage yield, even though seeding rate of WWG was 50% lower in these forage swards, suggesting that there is little forage yield penalty for including additional species/functional groups in the forage swards.

The legumes had lower plant densities than the grasses, which reduced their effects on the forage sward. Legume abundance may increase with moderate grazing, where forbs increase as grasses decrease (Bai et al. 2001). Purple prairie clover is known to have high protein, low fibre content, and is rated as a fair forage sward that increases under moderate grazing but suffers when overgrazed (Schellenberg and Banerjee 2002; McGraw et al. 2004; Tannas 2004; Stubbendieck et al. 2011). Prairie clovers require higher temperatures than grasses to initiate germination (Schellenberg and Henderson 2010), and are slow to establish with relatively low forage yield (Schellenberg and Banerjee 2002; Molano-Flores et al. 2011). Legume impact on soil nitrogen was not detectable within the first two years of being seeded. However, as the clovers become more established the benefits of including them may become more evident as more nitrogen is fixed into the soil.

The legumes and $C_4$ grasses were the least productive swards, which may be due to the exceptionally high precipitation levels during 2010 and 2011 and the cooler temperatures during the seeding year. Perennial species tend to invest in roots before shoots, especially in arid environments (Weaver 1958), therefore, full establishment and maximum forage yield may require more than two years for many of these species that do not exhibit the competitive life strategy displayed by WWG (Jefferson et al. 2002). $C_4$ grasses were included in part for their drought resistance which was not evident in such wet years. Benefits to including them may be more obvious in dryer years, where WWG may prove to be less successful. Additionally, rangeland plant diversity can improve the nutritional quality and palatability of forage by

providing a mixed diet, thereby increasing weight gain in large herbivores (Holechek et al. 2004; Wang et al. 2010).

More diverse forage swards may provide a more reliable source of forage yield even in years with very different environmental conditions (Lehman and Tilman 2000; Loreau and de Mazancourt 2013). In our study, species that benefited from the wet year ($C_3$ grasses) and rapidly established (WWG) were highly productive; however, in a more typical year, drought tolerant species may compensate for the $C_3$ grasses which become dormant in hot, dry conditions. Thus, forage swards of native perennial species have high potential for long-term production stability despite environmental variability, as diversity increases the reliability that the community will have long-term average performance (Yachi and Loreau 1999). Data collection should be maintained for these experimental plots to assess long-term variation, and to compare the community dynamics at the establishment phase to those at a more mature stage.

## 2.6 Conclusions

Seed of species native to the Canadian Mixed Grass Prairie is commercially available, but little information is available on appropriate multispecies forage swards for prairie restoration and seeded pastures. Fast growing and highly competitive species dominate biomass production in the early establishment phase of the community, however the inclusion of less productive species in the forage sward carries little penalty for pasture productivity or nutritional value. Less productive species thus should be included in pasture mixes when they bring beneficial traits (i.e. higher tolerance to grazing, drought resistance) to the restored forage sward. Including these traits carries little penalty under good growing conditions, and provides “insurance” for less optimal years. This work provides insight in forage sward development at the early establishment

stage; additional work is required to determine species impacts for well established forage swards.

## 2.7 References


**Acton, D. F. and Ellis, J. G. 1978**. The soils of the Saskatoon map area 73B Saskatchewan. Saskatchewan Institute of Pedology, Extension Division, University of Saskatchewan, Saskatoon, SK. Extension Publ. 306. 34 pp.

**Akaike, H. 1974**. A new look at the statistical model identification. IEEE T. Automat. Contr. **19**: 716-723.

**Ayers, K. W., Acton, D. F. and Ellis, J. G. 1985**. The soils of the Swift Current map area 72J Saskatchewan. Saskatchewan Institute of Pedology, Extension Division, University of Saskatchewan, Saskatoon, SK. Extension Publ. 481. 48 pp.

**Bahm, M. A., Barnes, T. G. and Jensen, K. C. 2011**. Restoring native plant communities in Smooth Brome (*Bromus inermis*)-dominated grasslands. Invasive Plant Sci. Manag. **4**: 239-250.

**Bai, Y., Abouguendia, Z. and Redmann, R. E. 2001**. Relationship between plant species diversity and grassland condition. J. Range Manag. **54**: 177-183.

**Branson, F. A. 1953**. Two new factors affecting resistance of grasses to grazing. J. Range Manag. **6**: 165-171.

**Brooker, R. W., Maestre, F. T., Callaway, R. M., Lortie, C. L., Cavieres, L. A., Kunstler, G., Liancourt, P., Tielbörger, K., Travis, J. M. J., Anthelme, F., Armas, C., Coll, L., Corcket, E., Delzon, S., Forey, E., Kikvidze, Z., Olofsson, J., Pugnaire, F., Quiroz, C. L., Saccone, P., Schiffers, K., Seifan, M., Touzard, B. and Michalet, R. 2008**. Facilitation in plant communities: the past, the present, and the future. J. Ecol. **96**: 18-34.

**Buxton, D. R. 1996**. Quality-related characteristics of forages as influenced by plant environment and agronomic factors. Anim. Feed Sci. Technol. **59**: 37-49.

**Callaway, R. M. 1995**. Positive interactions among plants. Bot. Rev. **61**: 306-349.

**Cooke, T. G. 1972**. Comparative nutritive values of forbs, grasses and shrubs. Pages 303-310 i*n* C.M. McKell, J.P. Blaisdell and J.R. Goodin (eds.). Wildland shrubs: Their biology and utilization. USDA forest service GTR INT-1.

**Crawley, M. J. 2007**. The R Book. John Wiley & Sons, Chichester, U. K.

**Díaz, S. and Cabido, M. 2001**. Vive la différence: plant functional diversity matters to ecosystem processes. Trends Ecol. Evol. **16**: 646-655.

**Dyksterhuis, E. J. 1949**. Condition and management of range land based on quantitative ecology. J. Range Manag. **2**: 104-115.

**Hamm, J. W., Radford, F. G. and Halstead, E. H. 1970**. The simultaneous determination of nitrogen, phosphorous and potassium in sodium bicarbonate extracts of soils. Pages 65-69 *in* Advances in Automatic Analysis: Technicon International Congress, New York. Vol II. Industrial Analysis.

**Hammermeister, A. M., Naeth, M. A., Schoenau, J. J. and Biederbeck, V. O. 2003**. Soil and plant response to wellsite rehabilitation on native prairie in southeastern Alberta, Canada. Can. J. Soil Sci. **83**: 507-519.

**Holechek, J. L., Pieper, R. D. and Herbel, C. H. 2004**. Range animal nutrition. Pages 265-316 *in* Range management: Principles and practices. 5th Ed. Prentice-Hall, Inc. Englewood Cliffs, New Jersey.

**Hooper, D. U., Chapin, F. S., Ewel, J. J., Hector, A., Inchausti, P., Lavorel, S., Lawton, J. H., Lodge, D. M., Loreau, M., Naeem, S., Schmid, B., Setala, H., Symstad, A. J., Vandermeer, J. and Wardle, D. A. 2005**. Effects of biodiversity on ecosystem functioning: A consensus of current knowledge. Ecol. Monogr. **75**: 3-35.

**Jefferson, P. G., Iwaasa, A. D., Schellenberg, M. P. and McLeod, J. G. 2005**. Re-evaluation of native plant species for seeding and grazing by livestock on the semiarid prairie of western Canada. Prairie Forum. **30**: 85-106.

**Jefferson, P. G., McCaughey, W. P., May, K., Woosaree, J., MacFarlane, L. and Wright, S. M. 2002**. Performance of American native grass cultivars in the Canadian prairie provinces. Native Plants Journal. **3**: 24-33.

**Jefferson, P. G., McCaughey, W. P., May, K., Woosaree, J. and McFarlane, L. 2004**. Forage quality of seeded native grasses in the fall season on the Canadian prairie provinces. Can. J. Plant Sci. **84**: 503-509.

**Lehman, C. L. and Tilman, D. 2000**. Biodiversity, stability, and productivity in competitive communities. Am. Nat. **156**: 534-552.

**Loreau, M. and de Mazancourt, C. 2013**. Biodiversity and ecosystem stability: a synthesis of underlying mechanisms. http://dx.doi.org/10.1111/ele.12073.

**McGraw, R. L., Shockley, F. W., Thompson, J. F. and Roberts, C. A. 2004**. Native legume species. Native Plants Journal. **5**: 152-159.

**Molano-Flores, B., Coons, J. M. and Cunningham, J. B. 2011**. Germination of two seed types from three *Dalea* species. Castanea. **76**: 266-271.

**Monsen, S. B., Stevens, R. and Shaw, N. 2004**. Chapter 18. Grasses. Pages 295-424 *in* Restoring western ranges and wildlands, vol. 2. USDA Forest Service Gen. Tech. Rep. RMRS-GTR-136.

**Muir, J. P., Pitman, W. D. and Foster, J. L. 2011**. Sustainable, low-input, warm-season, grass-legume grassland mixtures: mission (nearly) impossible? Grass Forage Sci. **66**: 301-315.

**National Research Council. 2000**. Nutrient requirements of beef cattle. 7th rev. ed. 1996 (updated 2001). Subcommittee on Beef Cattle Nutrition, Committee on Animal Nutrition, Board on Agriculture, National Research Council. National Academic Press, Washington, DC. 381 pp.

**Noel, R. J. and Hableton, L. G. 1976**. Collaborative study of semi-automated method for determination of crude protein in animal feeds. Assoc. Off. Anal. Chem. **59**: 134-140.

**Oelmann, Y., Wilcke, W., Temperton, V. M., Buchmann, N., Roscher, C., Schumacher, J., Schulze, E. D. and Weisser, W. W. 2007**. Soil and plant nitrogen pools as related to plant diversity in an experimental grassland. Soil Sci. Soc. Am. J. **71**: 720-729.

**Prairie Conservation Action Plan Partnership. 2005**. Saskatchewan prairie conservation action plan 2003-2008. Pp 46.

**Qian, P. and Schoenau, J. J. 2002**. Practical applications of ion exchange resins in agricultural and environmental soil research. Can. J. Soil Sci. **82**: 9-21.

**Richards, R. T., Chambers, J. C. and Ross, C. 1998**. Use of native plants on federal lands: Policy and practice. J. Range Manag. **51**: 625-632.

**Schellenberg, M. P. 2008**. Biomass yield differences for introduced versus native grasses in mono- and poly- cultures in southwestern Saskatchewan. Abstract in: "Building Bridges:

Grasslands to Rangelands". SRM-AFGC AGM 25-31 Louisville, KY. CD. Paper No. 2138.

**Schellenberg, M. P. and Banerjee, M. R. 2002**. The potential of legume-shrub mixtures for optimum forage production in southwestern Saskatchewan: A greenhouse study. Can. J. of Plant Sci. **82**: 357-363.

**Schellenberg, M. P., Biligetu, B. and Iwaasa, A. D. 2012**. Species dynamic, forage yield, and nutritive value of seeded native plant mixtures following grazing. Can. J. of Plant Sci. **92**: 699-706.

**Schellenberg, M. P. and Henderson, D. 2010**. Germination temperature characteristics of three Dalea spp. with overlapping ranges in the Canadian prairies. Abstract in: "Working landscapes, SRM and WSSA Joint AGM". Denver, CO. C-29.

**Simmers, S. M. and Galatowitsch, S. M. 2010**. Factors affecting revegetation of oil field access roads in semiarid grassland. Restor. Ecol. **18**: 27-39.

**Smith, R. S., Thome, S. and Randall, R. 1988**. Nitragin Brand Inoculants. Technical Bulletin No. 102. 8 pp.

**Stubbendieck, J. L., Hatch, S. L. and Bryan, N. M. 2011**. North American wildland plants: a field guide. University of Nebraska Press, Lincoln.

**Symstad, A. J., Chapin, F. S., Wall, D. H., Gross, K. L., Huenneke, L. F., Mittelbach, G. G., Peters, D. P. C. and Tilman, D. 2003**. Long-term and large-scale perspectives on the relationship between biodiversity and ecosystem functioning. Bioscience. **53**: 89-98.

**Tannas, K. 2004**. Common plants of the western rangelands: Forbs. Vol 3. Alberta Agriculture, Food and Rural Development. Pp 505.

**Temperton, V. M., Mwangi, P. N., Scherer-Lorenzen, M., Schmid, B. and Buchmann, N. 2007**. Positive interactions between nitrogen-fixing legumes and four different neighbouring species in a biodiversity experiment. Oecologia. **151**: 190-205.

**Tilman, D., Reich, P. B., Knops, J., Wedin, D., Mielke, T. and Lehman, C. 2001**. Diversity and productivity in a long-term grassland experiment. Science. **294**: 843-845.

**United States Department of Agriculture National Resources Conservation Service Plant Materials Program. 2002**. Western Wheatgrass *Pascopyrum smithii* (Rydb.) Scribn. http://plants.usda.gov/java/profile?symbol=PASM. 31 July 2012. USDA Forest Service Gen. Tech. Rep. RMRS-GTR-136.

**Wang, L., Wang, D. L., He, Z. B., Liu, G. F. and Hodgkinson, K. C. 2010**. Mechanisms linking plant species richness to foraging of a large herbivore. J. Appl. Ecol. **47**: 868-875.

**Wang, Z. and Schellenberg, M. P. 2012**. Drought and N addition in a greenhouse experiment: blue grama and western wheatgrass. J. Agr. Sci. Tech. B. **2**: 29-37.

**Weaver, J. E. 1958**. Summary and interpretation of underground development in natural grassland communities. Ecol. Monogr. **28**: 55-78.

**Weigelt, A., Weisser, W. W., Buchmann, N. and Scherer-Lorenzen, M. 2009**. Biodiversity for multifunctional grasslands: equal productivity in high-diversity low-input and low-diversity high-input systems. Biogeosciences. **6**: 1695-1706.

**Whitbread, A. M., Hall, C. A. and Pengelly, B. C. 2009**. A novel approach to planting grass-legume pastures in the mixed farming zone of southern inland Queensland, Australia. Crop Pasture Sci. **60**: 1147-1155.

**Yachi, S. and Loreau, M. 1999**. Biodiversity and ecosystem productivity in a fluctuating environment: The insurance hypothesis. Proc. Natl. Acad. Sci. USA. **96**: 1463-1468.

**Zhang, S. and Lamb, E. G. 2011**. Plant competitive ability and the transitivity of competitive hierarchies change with plant age. Plant Ecol. **213**: 15-23.

## Chapter 3 preamble

This chapter investigates whether the factors identified as influencing productivity in the are consistent under water-limited conditions, or if different community characteristics become important. Two sets of communities were planted, the second set of the communities were subjected to a drought treatment. The reduced soil moisture reduced the overall productivity of the communities in the drought treatment. In this analysis, all multi-group structural model equation models relating community composition to productivity had adequate fit, except for evenness. The results of this chapter demonstrate that the high productivity communities are likely to remain the best performers under moderate drought conditions.

This chapter relates to the overall thesis by addressing the study objective 2) to determine how water limitation impacted above-ground productivity, and if it altered the predictive ability of the community characteristics resulting in a different optimum community required under drought stress.

# 3 LIMITED EFFECTS OF MODERATE DROUGHT ON COMPLEX MIXTURES OF NATIVE GRASS AND LEGUME SPECIES

## 3.1 Abstract

Native species have the potential to provide non-invasive, productive, drought-resistant, perennial seeded pasture or mixed–grass rangelands. Little is known, however, about optimizing native seed mixes for both maximum productivity and drought resistance. Community productivity may be a function of community characteristics or composition (including species and functional group richness, evenness, and species and functional group identity). We assessed the effect of a moderate drought on productivity using two sets of the same communities that varied across the community characteristics, where one set of communities was subjected to water-limitation. The seven native perennial species used in this experiment were: nodding brome [*Bromus anomalus* (Coult.)], blue bunch wheatgrass [*Pseudoregneria spicata* (Pursh)], western wheatgrass [*Pascopyrum smithii* (Rydb.)], side oats grama [*Bouteloua curtipendula* (Michx.)], little blue stem [*Schizachyrium scoparium* (Michx.)], purple prairie clover [*Dalea purpurea* (Vent.)], and white prairie clover [*Dalea candida* (Willd.)]. We used multi-group structural equation modeling to test whether a moderate drought affected the relative importance of each of the above characteristics in determining community productivity. There was a trend for lower above ground productivity in drought conditions, but drought did not change the influence of community characteristics on productivity, implying adequate drought tolerance of these species. The consistency of the relative productivity indicates that the optimal communities identified will likely be stable under moderate drought conditions.

## 3.2 Introduction

There is a growing interest in the use of native perennial species for seeded rangeland and reclamation following disturbance as native species may increase carbon sequestration, improve wildlife habitat, lower agronomic inputs, and extend the grazing season, and provide more reliable forage in drought conditions (Symstad et al. 2003; Jefferson et al. 2005; Prairie Conservation Action Plan Partnership 2005; Weigelt et al. 2009; Muir et al. 2011; Chapter 2: Mischkolz et al. in press). Native perennial mixtures have the potential to be as productive as tame monocultures in a greater range of environmental conditions (Schellenberg 2008). However, maintaining forage quantity and quality is challenging in grazing systems where herbivory and/or drought stresses plants and potentially eliminates desirable species from the community (Heady and Child 1994; Gerrish 2001). There is some evidence that production stability under drought pressure can be increased through community diversity (Frank and Mcnaughton 1991; Tilman and Downing 1994). This stability is likely a result of the differing ability of plant species to cope with water stress. Mixtures are expected to be more stable over the growing season and across years than monocultures because fluctuations in one species' forage yield would be compensated for by other species in the community (Doak et al. 1998; Tilman 1999; Lhomme and Winkel 2002). Thus, mixtures of native perennial species have high potential for long-term production stability despite environmental variability, as diversity increases the reliability that the community will have long-term performance (Yachi and Loreau 1999; Schellenberg 2008).

Productivity is important in a rangeland setting for providing increased forage material, and has been suggested as an indicator of ecological function (Hooper et al. 2005; Kirwan et al. 2007). Increases in diversity, including measure of species richness, functional group richness,

and relative abundance (evenness) have been found to increase productivity (Balvanera et al. 2006; Kirwan et al. 2007; Weigelt et al. 2009; Chapter 4). Drought conditions may change which community characteristics most strongly influence productivity. This is important in arid and semi-arid grasslands where plant growth is limited by water availability (Herbel et al. 1972; Tilman and Elhaddi 1992; Johnson and Asay 1993). Southwestern Saskatchewan is predicted to become more arid under global warming climate change scenarios (Cutforth 2000), with greater variation in rainfall patterns (Easterling et al. 2000). Determining how drought stress influences productivity for complex communities is critical to identifying a stable, highly productive forage sward mixture for this region. In this study, the objectives were to determine 1) how water limitation impacted above-ground productivity; and 2) if it altered the predictive ability of the community characteristics resulting in a different optimum community required under drought stress.

## 3.3 Material and methods

We conducted a greenhouse experiment to study the response of complex communities to moderate drought conditions using seven native perennial species. These species are nodding brome [*Bromus anomalus* (Coult.)], blue bunch wheatgrass [*Pseudoregneria spicata* (Pursh)], western wheatgrass [*Pascopyrum smithii* (Rydb.)], side oats grama [*Bouteloua curtipendula* (Michx.)], little blue stem [*Schizachyrium scoparium* (Michx.)], purple prairie clover [*Dalea purpurea* (Vent.)], and white prairie clover [*Dalea candida* (Willd.)]. The species selected have high agronomic potential (reasonable plant size, plant nutritional quality, cost, availability, and the ability to work with conventional machinery), and a broad native geographic range spanning western Canada. These species are currently being evaluated in breeding programs, a long term

multi-species field trial, and greenhouse trials (Chapter 2; Chapter 4; Schellenberg 2008; Zhang and Lamb 2011).

We conducted this experiment in the University of Saskatchewan Agriculture Greenhouse which has an 18 h photoperiod supplemented with electric lighting when needed, 6 h dark, average day air temperature of 23°C (max = 36°C, min = 17°), average night air temperature of 19°C (max= 23°C, min = 17°), and an average relative humidity of 55% (max = 93%, min = 16%)). In the pots, we used a 4:1 mixture of potting soil to topsoil. Topsoil was included in the pots to ensure the microorganisms present in the semi-arid grassland were also present in the greenhouse experiment to allow for possible synergistic associations. The topsoil was a brown Swinton sandy loam from an experimental forage field near Swift Current, SK at the Swift Current Semi-Arid Prairie Agricultural Research Center (a field site of the field trial study; soil properties in (Schellenberg and Banerjee 2002)).

### *3.3.1 Experimental design*

In a community (pot) with 21 plants of up to seven species, there are 296, 010 combinations that represent the pool of possible communities varying in species richness, evenness, and composition. We selected 158 communities from the pool of possible communities using a combination of stratified random selection within each level of species richness, and fully random selection. We selected all the monocultures (n=7) as well as 10 community mixtures from each of the other levels of species richness (2 to 7) to ensure we captured the full range of species richness (n=60). We randomly selected 91 additional community mixtures from the remaining pool for a total of 158 pots (the available space). We planted two sets of these communities, one set as the control, and the second set as a water limited treatment.

Within each selected community, we randomly assigned individual plants to the 21 planting positions in the pot (see Appendix Figure 6.2), and transplanted 10 day old seedlings into their positions. Two weeks after planting, we assessed seedling health and replaced dead seedlings to ensure experimental communities represented the goal communities. We assessed seedlings again after another two weeks and did not replace dead seedlings again as differences in age between plants would be too large; instead, we recorded the live plants in each pot as the initial communities. We harvested above ground biomass after 12 weeks of growth. Plants were clipped at the base, dried in an oven at 55 degrees Celsius for 48 hours, and weighed. We did not include pots with more than 6 seedling mortalities in our analyses as communities no longer resembled the goal communities. This criterion resulted in the removal of four pots from the one treatment and their paired pot from the other treatment, resulting in a total sample size of 154 pots per treatment.

### *3.3.2 Water regime*

The water regime began after the seedlings were allowed to establish for 4 weeks. Communities in the control treatment were watered every 4 days, and communities in the water limited treatment were watered every 8 days (50% increase in length of dry intervals between watering). Soil moisture was measured for every pot using a Time-domain Reflectometer probe (TDR probe; Trase Systems I) to confirm that the water limited treatment had successfully created moderate drought conditions. TDR measurements were taken before watering near the end of the experiment 1) 4 days after watering both the control and water-limited treatments, and 2) 8 days after watering only the control treatment. Paired t-tests were used to confirm that soil moisture was lower in the water-limited treatment for both time points sampled: a) half drought intensity (20% less water, $t = 5.5386$, $df = 152$, p-value $<0.0001$), b) full drought intensity (66%

less water, t=17.15, df =147, p<0.0001) (Figure 3.1). Sample sizes differed for the tests because of missing data (six pots from the sample at half drought intensity (n=148), and one pot from the sample at full drought intensity (n=153)).

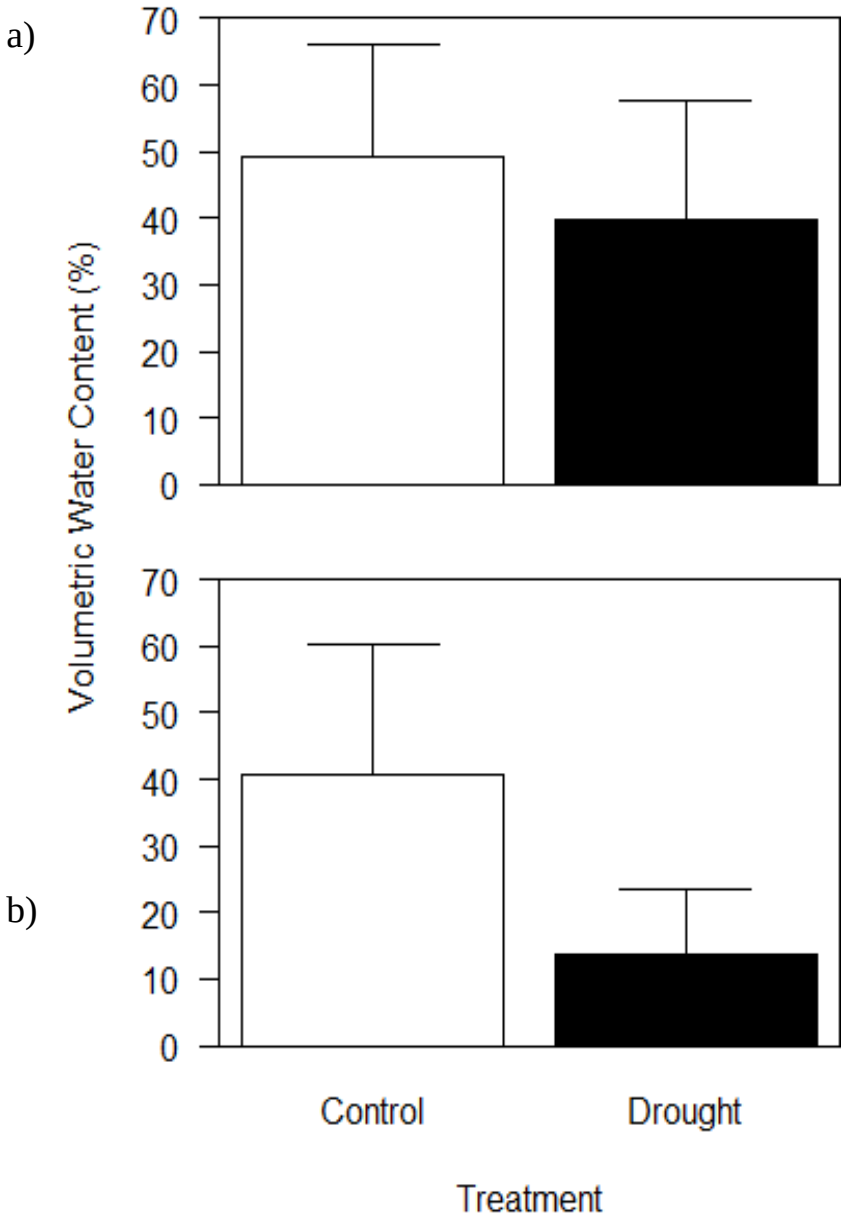


**Figure 3.1.** Soil moisture was significantly lower in the water-limited treatment at two points in the watering regime at the end of the experiment. Error bars are one standard deviation.

### *3.3.3 Statistical analysis*

The effect of reduced soil moisture on productivity was assessed using a paired t-test where communities with the same composition were paired to control for the effects of community composition on productivity. Tests were performed using the R statistical package (R Development Core Team 2011).

The effects of drought on the relationships between plant community characteristics and productivity was assessed for seven major community characteristics (Table 3.1). The goal here was to assess whether drought altered the relationships between community characteristics and productivity. For more detail on variables and rationale of *a priori* theories used in models see Tables 3.1, and Appendix Table 6.5). Multiple-group structural equation models (SEM) were used to test whether the direct causal effects of community characteristics on productivity differed between drought and control treatments (Grace 2006; Lamb et al. 2011). Initial models linking variables from each *a-priori* theory to productivity were developed. In models with multiple explanatory variables, these variables were inter-correlated as a result of our experimental design restricting the community to 21 individuals. For example, a community that has one species with an abundance of 20 individuals can only contain one individual of one other species (thereby limiting species and functional group composition, and evenness). The effect of the water limited treatment on the strengths of relationships (path coefficients) within each of the SEM models was assessed using multi-group SEM analyses, where the models are fit to the data of both treatments separately (group 1: control, and group 2: water limited). Models that adequately fit with all model parameters constrained to be equal between groups indicate that the drought treatment did not change that explanatory variables' effect on productivity. The SEM models were fit using Amos 18.0 (Amos Development Corporation, Crawfordville, FL, USA).

**Table 3.1.** Summary model fit for the seven *a priori* models in multi-group structural equation model, where group 1: control, and group 2: water limited.

| Model | References for rational | $X^2$ | df | p-value | $R^2$ | CFI | RMSEA (90% CI) |
|---|---|---|---|---|---|---|---|
| A Species richness | Increase in productivity with increasing species richness as more traits become present and increased stability due to increased redundancy; (Lehman and Tilman 2000; Kirwan et al. 2007) | 1.465 | 3 | 0.690 | 0.080 | 1.000 | 0.000 (0.000-0.073) |
| B Evenness | Increase in productivity with increasing evenness due to increasing numbers of interactions (Kirwan et al. 2007) | 0.11 | 3 | 0.991 | 0.00 | 1.000 | 0.000 (0.000-0.997) |
| C Functional group richness | Increase in productivity with increasing functional group richness as more traits become present and increased stability due to increased redundancy at a more general scope than species richness (Reich et al. 2004; Balvanera et al. 2006) | 1.955 | 3 | 0.582 | 0.080 | 1.000 | 0.000 (0.000-0.082) |
| D Number of plant of each species | Increase in productivity with increased abundance of species suited to environmental conditions. (Tilman et al. 1996; Hooper et al. 2005) | 13.34 | 36 | 1.000 | 0.093 | 1.000 | 0.000 (0.000-1.000) |
| E Number of plants in each functional group | Increase in productivity with increased abundance of functional groups suited to environmental conditions. (Hooper et al. 2005) | 6.057 | 10 | 0.810 | 0.081 | 1.000 | 0.000 (0.000-0.039) |
| F Species presence | Increase in productivity with the presence of species in the community due to high complimentarity or facilitation of that species to the other plants. (Hooper et al. 2005; Johnson et al. 2008) | 12.94 | 36 | 1.000 | 0.127 | 1.000 | 0.000 (0.000-0.000) |
| G Functional group presence | Increase in productivity with the presence of functional groups in the community due to high complimentarity or facilitation of that functional group to the other plants. (Hooper et al. 2005) | 3.571 | 10 | 0.965 | 0.157 | 1.000 | 0.000 (0.000-0.998) |

*see Appendix Table 6.5-6.7 for more detail on models, and results

## 3.4 Results and discussion

The productivity of water-limited communities trended towards a significant reduction compared to the control (-5.7%, t=1.96, df=153, p=0.052, Figure 3.2). All models had adequate fit in the multi-group analysis with all path coefficients fully constrained to be equal between groups (Table 3.1; and in Appendix Tables 6.6 and 6.7). While productivity was reduced to some degree, the response of the plants to the moderate drought did not change the identity or relative importance of community characteristics driving productivity. This indicates that these species and communities are robust to moderate drought, as may be expected from species that are adapted to the semi-arid grassland region (Turner 1986). These results may not be consistent as the perennial plants age, however it is not feasible to assess multi-year effects on perennial plants in a greenhouse experiment due to the pots becoming root bound. Long term effect of drought should be assessed in the field. Productivity was slightly reduced in the water limited conditions within this growing period, but the relative productivity of communities remained consistent, indicating that the optimal communities will likely be stable under moderate drought conditions, at least within the early establishment phase of these species. Additionally, longer-term effects of the drought conditions are more difficult to assess but may be important as a lag effect of drought has been shown to negatively impact the plant vigor in the next season (Herbel et al. 1972).

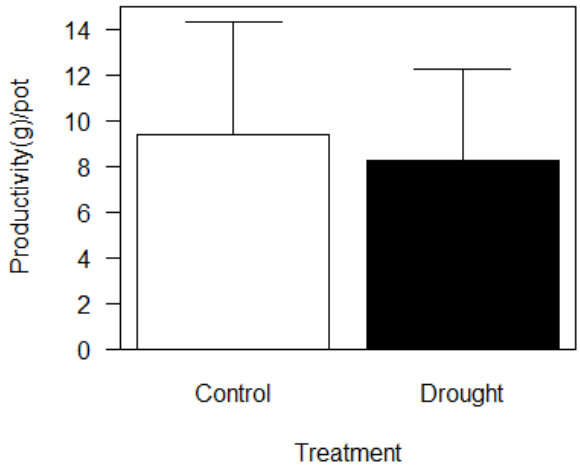


**Figure 3.2.** Overall productivity response to water-limitation displays a trend towards a significant reduction (p=0.052, 5.7 %), relative to the control. Error bars are one standard deviation.

The responses of the communities were consistent across the moderate drought treatment and the SEM models indicated that community characteristics significantly influenced productivity. Increases in species richness, functional group richness, the number of WWG individuals, and the presence of BWG and WWG in the community all significantly increase community productivity (Figure 3.3). As these relationships did not differ between control conditions and water-limited conditions, the relative advantage of high productivity communities remains under moderate drought conditions. This pattern is important and consistent for all models, despite the low $R^2$ values. Therefore, the water limited treatment only affected total productivity and did not change the estimated influence of predictor variables for community productivity.

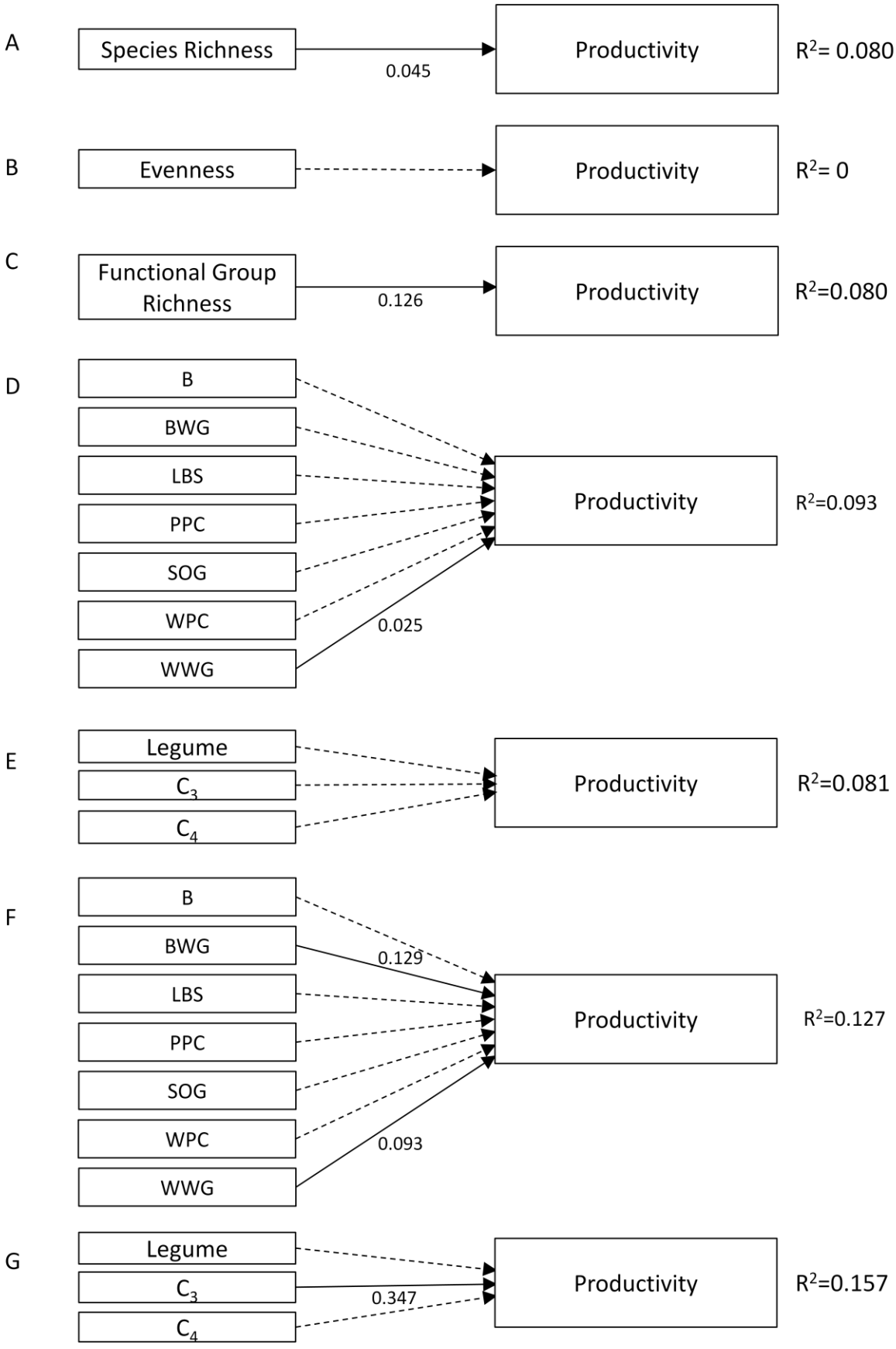
A
Species Richness
0.045
Productivity
R2= 0.080
B
Evenness
Productivity
R2= 0
C
Functional Group Richness
0.126
Productivity
R2=0.080
D
B
BWG
LBS
PPC
SOG
WPC
WWG
0.025
Productivity
R2=0.093
E
Legume
C3
C4
Productivity
R2=0.081
F
B
BWG
LBS
PPC
SOG
WPC
WWG
0.129
0.093
Productivity
R2=0.127
G
Legume
C3
C4
0.347
Productivity
R2=0.157

**Figure 3.3.** Structural equation models of competing models used in multi-group analyses. The boxes represent observed variables and single headed arrows indicate direct relationships. Double headed arrows (inter-correlations) are not displayed. All models had adequate fit. Standardized path coefficients are displayed for significant ($P<0.05$) paths. Non-significant paths are dotted. B, nodding brome, *Bromus anomalus*; BWG, blue bunch wheatgrass, *Pseudoregneria spicata*; LBS, little blue stem, *Schizachyrium scoparium*; PPC, purple prairie clover, *Dalea purpurea*; SOG, side oats grama, *Bouteloua curtipendula*; WPC, white prairie clover, *Dalea candida*; WWG, western wheatgrass, *Pascopyrum smithii*; $C_3$, cool season grasses; $C_4$, warm season grasses. *see Appendix Table 6.5-6.7 for more detail

## 3.5 References


**Balvanera, P., Pfisterer, A. B., Buchmann, N., He, J. S., Nakashizuka, T., Raffaelli, D. and Schmid, B. 2006**. Quantifying the evidence for biodiversity effects on ecosystem functioning and services. Ecol. Lett. **9**: 1146-1156.

**Cutforth, H. W. 2000**. Climate change in the semiarid prairie of southwestern Saskatchewan: Temperature, precipitation, wind, and incoming solar energy. Can. J. Soil Sci. **80**: 375-385.

**Doak, D. F., Bigger, D., Harding, E. K., Marvier, M. A., O'Malley, R. E. and Thomson, D. 1998**. The statistical inevitability of stability diversity relationships in community ecology. The American Naturalist. **151**: 264-276.

**Easterling, D. R., Meehl, G. A., Parmesan, C., Changnon, S. A., Karl, T. R. and Mearns, L. O. 2000**. Climate extremes: Observations, modeling, and impacts. Science. **289**: 2068-2074.

**Frank, D. A. and Mcnaughton, S. J. 1991**. Stability increases with diversity in plant-communities: Empirical evidence from the 1988 Yellowstone drought. Oikos. **62**: 360-362.

**Gerrish, J. 2001.** Species stability in diverse pasture mixtures. Pages 212-216 *in* T. Terrill, ed. American Forage and Grassland Council, Vol 10, Proceedings. Amer Forage & Grassland Council, Georgetown.

**Grace, J. B. 2006**. Structural equation modeling and natural systems. Cambridge University Press, Cambridge.

**Heady, H. F. and Child, R. D. 1994**. Rangeland Ecology and Managment. Westview Press, Boulder, Colorado.

**Herbel, C. H., Ares, F. N. and Wright, R. A. 1972**. Drought effects on a semidesert grassland range. Ecology. **53**: 1084-1093.

**Hooper, D. U., Chapin, F. S., Ewel, J. J., Hector, A., Inchausti, P., Lavorel, S., Lawton, J. H., Lodge, D. M., Loreau, M., Naeem, S., Schmid, B., Setala, H., Symstad, A. J., Vandermeer, J. and Wardle, D. A. 2005**. Effects of biodiversity on ecosystem functioning: A consensus of current knowledge. Ecol. Monogr. **75**: 3-35.

**Jefferson, P. G., Iwaasa, A. D., Schellenberg, M. P. and McLeod, J. G. 2005**. Re-evaluation of native plant species for seeding and grazing by livestock on the semiarid prairie of western Canada. Prairie Forum. **30**: 85-106.

**Johnson, D., Phoenix, G. K. and Grime, J. P. 2008**. Plant community composition, not diversity, regulates soil respiration in grasslands. Biology Letters. **4**: 345-348.

**Johnson, D. A. and Asay, K. H. 1993**. Viewpoint: selection for improved drought response in cool-season grasses. J. Range Manag. **46**: 194-202.

**Kirwan, L., Luescher, A., Sebastia, M. T., Finn, J. A., Collins, R. P., Porqueddu, C., Helgadottir, A., Baadshaug, O. H., Brophy, C., Coran, C., Dalmannsdottir, S., Delgado, I., Elgersma, A., Fothergill, M., Frankow-Lindberg, B. E., Golinski, P., Grieu, P., Gustavsson, A. M., Hoglind, M., Huguenin-Elie, O., Iliadis, C., Jorgensen, M., Kadziuliene, Z., Karyotis, T., Lunnan, T., Malengier, M., Maltoni, S., Meyer, V., Nyfeler, D., Nykanen-Kurki, P., Parente, J., Smit, H. J., Thumm, U. and Connolly, J. 2007**. Evenness drives consistent diversity effects in intensive grassland systems across 28 European sites. J. Ecol. **95**: 530-539.

**Lamb, E. G., Shirtliffe, S. J. and May, W. E. 2011**. Structural equation modeling in the plant sciences: An example using yield components in oat. Can. J. Plant Sci. **91**: 603-619.

**Lehman, Clarence L. and Tilman, D. 2000**. Biodiversity, stability, and productivity in competitive communities. Am. Nat. **156**: 534-552.

**Lhomme, J. p. and Winkel, T. 2002**. Diversity stability relationships in community ecology: Re-examination of the portfolio effect. Theor. Popul. Biol. **62**: 271-279.

**Mischkolz, J., Schellenberg, M. P. and Lamb, E. G. In press**. Early productivity and crude protein content of establishing forage swards composed of combinations of native grass and legume species in mixed-grassland ecoregions. Can. J. Plant Sci.

**Muir, J. P., Pitman, W. D. and Foster, J. L. 2011**. Sustainable, low-input, warm-season, grass-legume grassland mixtures: mission (nearly) impossible? Grass Forage Sci. **66**: 301-315.

**Prairie Conservation Action Plan Partnership. 2005**. Saskatchewan prairie conservation action plan 2003-2008. Pp 46.

**Reich, P. B., Tilman, D., Naeem, S., Ellsworth, D. S., Knops, J., Craine, J., Wedin, D. and Trost, J. 2004**. Species and functional group diversity independently influence biomass accumulation and its response to CO2 and N. Proc. Natl. Acad. Sci. U. S. A. **101**: 10101-10106.

**Schellenberg, M. P. 2008**. Biomass yield differences for introduced versus native grasses in mono- and poly- cultures in southwestern Saskatchewan. Abstract in: "Building Bridges: Grasslands to Rangelands". SRM-AFGC AGM 25-31 Louisville, KY. CD. Paper No. 2138.

**Symstad, A. J., Chapin, F. S., Wall, D. H., Gross, K. L., Huenneke, L. F., Mittelbach, G. G., Peters, D. P. C. and Tilman, D. 2003**. Long-term and large-scale perspectives on the relationship between biodiversity and ecosystem functioning. Bioscience. **53**: 89-98.

**Tilman, D. 1999**. The ecological consequences of changes in biodiversity: A search for general principles. Ecology. **80**: 1455-1474.

**Tilman, D. and Downing, J. A. 1994**. Biodiversity and Stability in Grasslands. Nature. **367**: 363-365.

**Tilman, D. and Elhaddi, A. 1992**. Drought and biodiversity in grasslands. Oecologia. **89**: 257-264.

**Tilman, D., Wedin, D. and Knops, J. 1996**. Productivity and sustainability influenced by biodiversity in grassland ecosystems. Nature. **379**: 718-720.

**Turner, N. 1986**. Adaptation to water deficits: A changing perspective. Funct. Plant Biol. **13**: 175-190.

**Weigelt, A., Weisser, W. W., Buchmann, N. and Scherer-Lorenzen, M. 2009**. Biodiversity for multifunctional grasslands: equal productivity in high-diversity low-input and low-diversity high-input systems. Biogeosciences. **6**: 1695-1706.

**Zhang, S. and Lamb, E. G. 2011**. Plant competitive ability and the transitivity of competitive hierarchies change with plant age. Plant Ecol. **213**: 15-23.

**Yachi, S. and Loreau, M. 1999**. Biodiversity and ecosystem productivity in a fluctuating environment: The insurance hypothesis. Proc. Natl. Acad. Sci. USA. **96**: 1463-1468.

## Chapter 4 preamble

There remains much to learn about features of plant communities that drive productivity, with many community characteristics and plant traits having been reported as influential. However, seed mixtures for restoration and seeded rangelands are not being developed using this knowledge as a predictive tool. Therefore, this next chapter compares the predictive power of the community characteristics and plant traits in the community using an initial experiment, and tests this tool using a confirmatory experiment. The strongest predictors of productivity were the presence and abundance of perennial $C_3$ grasses. Increases in species richness, functional group richness, and presence of $C_3$ grasses (particularly western wheatgrass) also increased productivity, likely because of the high early relative growth rate and strong competitive effect of western wheatgrass. Despite low $R^2$s, the confirmatory experiment demonstrated utility as a tool to separate low and high productivity communities; thus this new approach to constructing communities may be useful in screening species/communities for rangeland restoration.

This chapter relates to the overall thesis by addressing objective 3) to assess more complex mixtures by developing and applying a method (using community characteristics to predict productivity) to rapidly and systematically screen potential communities for high productivity. The same species assessed in the previous chapters were planted in complex mixtures in a greenhouse pot experiment.

# 4 ASSEMBLING PRODUCTIVE COMMUNITIES OF NATIVE GRASS AND LEGUME SPECIES: FINDING THE RIGHT MIX

## 4.1 Abstract

Native species have the potential to provide productive, drought-resistant rangelands. Little is known, however, about how to identify mixtures with optimal levels of productivity and stress resistance. Our objective was to develop empirical models that could predict optimal community compositions of seven native grasses and legumes from the pool of possible communities. We planted a selection of communities varying in species and functional group richness, evenness, and species and functional group identity in a stratified response surface design. Productivity was driven by species richness, and functional group richness of the community, the abundance and occurrence of particular species or functional groups, and plant traits within the community. The strongest predictors of productivity were the presence and abundance of perennial $C_3$ grasses. Increases in species richness, functional group richness, and presence of $C_3$s (particularly western wheatgrass) also increased productivity, likely because of the high early relative growth rate and strong competitive effect of western wheatgrass. We used the best predictive model to identify a set of predicted optimal communities, and tested those predictions in a confirmatory experiment. The observed relationship between predicted and observed productivity was positive and indicates that our equations predicted high productivity well. Therefore, we propose that this approach can be used as a general tool to screen species and potential community compositions mixtures when designing seed mixtures for ecological restoration.

## 4.2 Introduction

In natural grasslands, each species naturally coexists with hundreds of other species in complex communities. Commercial seed mixtures rarely contain more than a small number of species, often with haphazard ratios of the component species. Selected species may be native to the area, but all species are not necessarily present in any given community. Thus, there is no natural template for combining selected species into an optimal community and there is limited knowledge on how to compose a suitable species mixture. There remains much to learn about features of plant communities that drive optimal ecological function, with many community characteristics and plant traits having been reported as influential (i.e. Hooper et al. 2005). However, seed mixtures for restoration and seeded rangelands are not being developed using this knowledge. Therefore, identifying the features of a community that drive increased optimal ecological function and applying them as predictive tools may aid in screening species and community compositions. This may lead to mixtures that are more specifically designed to be stable and highly productive for the region.

High productivity is a critical ecological function in restored rangeland ecosystems (Hooper et al. 2005; Kirwan et al. 2007). High species richness, functional group richness, species evenness of the community, and diverse community compositions have been found to increase productivity (Loreau et al. 2001; Hooper et al. 2005; Balvanera et al. 2006; Kirwan et al. 2007; Weigelt et al. 2009; Lamb et al. 2011). The average trait values of the plants in the community, such as relative growth rate (RGR), specific leaf area (SLA), specific root length (SRL), and relative competitive ability, may also influence community productivity. High RGRs is generally found in species that have a competitive life strategy; whereas a lower RGR may correspond to species that have a stress-tolerant life strategy and do well in stable, nutrient

deficient habitats (Grime 1974; Grime and Hunt 1975). SLA is an indication of investment of carbon in leaves, where a low SLA indicates small thick leaves that require high investment relative to photosynthetic ability; conversely high SLA indicates large thin leaves that require less investment. High SLA is correlated with high RGR, large broad leaves, and aggressive, fast growing plants that are generally more palatable (Grime 1977). High SRL increases with nutrient and water acquisition ability (Aerts et al. 1991). SRL is a highly variable trait among species, where a plant may alter the arrangement of their roots or foraging strategies in the presence of other plants (but see Cahill 2003; Weigelt et al. 2005). The ability of a species to increase SRL in response to competition may also allow higher productivity.

Productivity is linked to the type and intensity of interactions between plants in a community where plants may either compete or complement each other in regard to resource acquisition and growth (Trenbath 1974; Miller 1994; Hooper et al. 2005; Sheehan et al. 2006; Lamb et al. 2011). Negative or competitive interactions between plants may lead to the loss of species from a community if there is a large disparity in relative competitive abilities. The competitive ability of a plant has two components, the ability to endure growth suppression by neighbours (competitive response; CR), and the ability to suppress neighbours (competitive effect; CE) (Goldberg et al. 1990; Goldberg 1996). Competitive effect ability is linked to size related plant traits (such as RGR) in high nutrient conditions, and root-related traits (such as SRL) in low nutrient conditions, whereas competitive response ability is a summary of a plant's ability to persist and avoid damage but does not necessarily link to specific plant traits (Wang et al. 2010). Positive or complimentary interactions between plants are due to combinations of traits or functional roles that are beneficial and can increase overall productivity; complementarity is most evident when species possess different traits (Callaway 1995; Hooper et al. 2005; Brooker et al. 2008). For

example, low soil nitrogen availability may limit grasses, but legume presence can alleviate nitrogen deficiencies (Bertness and Callaway 1994; Tilman 1997; Bruno et al. 2003; Muir et al. 2011). Complementarity may be critical to maintain productive communities in severe environments (Choler et al. 2001; but see Maestre et al. 2005; Callaway 2007). The most productive communities will have limited negative interactions and higher overall productivity.

There is a growing interest in the use of native perennial species for both seeded rangeland and reclamation, as native species may increase carbon sequestration, improve wildlife habitat, lower agronomic inputs, and extend the grazing season (Symstad et al. 2003; Jefferson et al. 2005; Prairie Conservation Action Plan Partnership 2005; Weigelt et al. 2009; Muir et al. 2011). Seven grass and legume species are undergoing evaluation for seeded pastures in the Mixed Grass Prairie ecoregion of Canada (Mischkolz et al. In Press), however, there is no natural template to identify the most productive combinations of the selected species. Here we assess complex mixtures of the species by developing and applying a method (using community characteristics to predict productivity) to rapidly and systematically screen potential communities for high productivity.

## 4.3 Materials and methods

We used greenhouse experiments to evaluate the productivity of communities assembled from seven grass and legume species. The seven selected species are *Pascopyrum smithii* (Western Wheatgrass, WWG), *Pseudoregeneria spicatum* (Bluebunch Wheatgrass, BWG), *Schizachyrium scoparius* (Little Blue Stem, LBS), *Bouteloua curtipendula* (Side Oat Grama, SOG), *Bromus anomalus* (Nodding Brome, B), *Dalea purpurea* (Purple Prairie Clover, PPC), and *Dalea candida* (White Prairie Clover, WPC). These species have high agronomic potential (reasonable

plant size, plant nutritional quality, cost, availability, and the ability to work with conventional machinery), and a broad native geographic range spanning western Canada. These species are currently being evaluated in breeding programs, are in the early phases of evaluation in a long term multi-species field trial (Chapter 2: Mischkolz et al. In press), and have a well understood competitive hierarchy (Zhang and Lamb 2011).

We conducted the experiments in the University of Saskatchewan Agriculture Greenhouse which has an 18 h photoperiod supplemented with electric lighting when needed, 6 h dark, average day air temperature of 23°C (max = 36°C, min = 17°), average night air temperature of 19°C (max= 23°C, min = 17°), and an average relative humidity of 55% (max = 93%, min = 16%)). In the pots, we used a 4:1 mixture of potting soil to topsoil. Topsoil was included in the pots to ensure the microorganisms present in the semi-arid grassland were also present in the greenhouse experiment to allow for possible synergistic associations. The topsoil was a brown Swinton sandy loam from an experimental forage field near Swift Current, SK at the Swift Current Semi-Arid Prairie Agricultural Research Center (a field site of the field trial study; soil properties in (Schellenberg and Banerjee 2002)).

### *4.3.1 Experimental design*

In a community (pot) with 21 plants of up to seven species, there are 296, 010 combinations that represent the pool of possible communities varying in species richness, evenness, and composition. We designed a series of three experiments to identify the most productive community configurations. The three experiments used communities drawn from the pool of possible communities, the details of which will be described in the following sections and included: a) an initial experiment to determine the characteristics (species richness, species

evenness, functional group richness, species abundance, functional group abundance, species occurrence, functional group occurrence, total relative growth rate, early relative growth rate, late relative growth rate, specific leaf area, specific root length, competitive effect, or competitive response) that best predicted productivity, b) a confirmatory experiment to assess if the best model from the initial experiment accurately predicted productivity, and c) an experiment to assess within community variation.

#### *4.3.1.1 Initial Experiment*

The initial experiment previously was used as the control treatment in the water limited experiment in Chapter 3. We selected 158 communities from the pool of possible communities using a combination of stratified random selection within each level of species richness, and fully random selection. We selected all the monocultures (n=7) as well as 10 community mixtures from each of the other levels of species richness (2 to 7) to ensure we captured the full range of species richness (n=60). We randomly selected 91 additional community mixtures from the remaining pool for a total of 158 pots (the available space).

Within each selected community, we randomly assigned individual plants to the 21 planting positions in the pot (see Appendix Figure 6.2), and transplanted 10 day old seedlings into their positions. Two weeks after planting, we assessed seedling health and replaced dead seedlings to ensure experimental communities represented the goal communities. We assessed seedlings again after another two weeks and did not replace dead seedlings again as differences in age between plants would be too large; instead, we recorded the live plants in each pot as the initial communities. We harvested above ground biomass after 12 weeks of growth. Plants were clipped at the base, dried in an oven at 55 degrees Celsius for 48 hours, and weighed. We did not include

pots with more than 6 seedling mortalities in our analyses as communities no longer resembled the goal communities. This removed four pots from the initial experiment.

#### *4.3.1.2 Confirmatory Experiment*

We tested if our predictive model linking community characteristics and productivity (see statistical analyses) in a confirmatory experiment by assessing a second set of communities and comparing observed and predicted productivity. We estimated the predicted productivity for all of the remaining possible communities in the pool using the linear equation from the best model developed for the initial experiment. To select communities for the confirmatory experiment, we ranked the pool of communities according to predicted productivity, and randomly selected seven communities from each of the $90^{th}$ and higher percentiles and similarly for the $1^{st}$ to $10^{th}$ percentiles for predicted productivity for a total of 140 pots. We stratified our community selection by percentile to ensure wide selection across the range of possible high and low productivity communities. We used the same experimental procedure as in the initial experiment. Two pots with more than six mortalities were removed from the confirmatory experiment.

#### *4.3.1.3 Within Community Variation*

We assessed the within community variation in a third experiment, as results for the initial experiment suggested substantial within community variation in productivity. Of the 140 communities selected for the confirmatory experiment, we randomly selected three communities predicted to have low productivity, and three communities predicted to have high productivity. We planted three additional replicates of each of these communities for a total of four replicates of each of the six communities. These communities had the same composition, but plants were randomly assigned to different positions within the pots. We used the same experimental

procedure as in the initial experiment. One pot with more than six mortalities was removed from the within community variation experiment.

#### *4.3.1.4 Trait data*

We measured several key leaf and root traits (specific leaf area, SLA; specific root length, SRL; and relative growth rate, RGR) for each species following published guidelines (Cornelissen et al. 2003; Levang-Brilz and Biondini 2003). We measured SLA and SRL for each species at two time points: early (day 55), and late (day 100), and RGR (biomass/#days) for each species during three time periods: total (day 0-100), early (day 0-55), and late (day 55-100) (Table 4.1). In addition, we used two measures of relative competitive ability for these species: adult competitive response (CR) and adult competitive effect (CE) values (Zhang and Lamb 2011, Table 4.1). We extrapolated species traits (SLA, SRL, RGR, CR, and CE) to the community level by multiplying the proportional abundance of each species in the community by the value of the trait for that species, and summing across species to obtain an overall value of that trait for the community (see Appendix Table 6.5 for more detail).

**Table 4.1.** Summary of specific leaf area (SLA; $cm^2/g$), specific root length (SRL; m/g), relative growth rates (RGR; g/day, Total = 0-100, Early = 0-55 days, Late = 55-100 days), and competitive response and effect for the seven species: B, nodding brome, *Bromus anomalus*; BWG, blue bunch wheatgrass, *Pseudoregneria spicata*; LBS, little blue stem, *Schizachyrium scoparium*; PPC, purple prairie clover, *Dalea purpurea*; SOG, side oats grama, *Bouteloua curtipendula*; WPC, white prairie clover, *Dalea candida*; WWG, western wheatgrass, *Pascopyrum smithii*.

| Species | SLA Day 55 | SLA Day 100 | SRL Day 55 | SRL Day 100 | Total RGR | Early RGR | Late RGR | Adult Competitive Response* | Adult Competitive Effect* |
|---|---|---|---|---|---|---|---|---|---|
| B | 230.09 | 188.14 | 40062.65 | 12644.12 | 0.4400 | 0.0944 | 0.8624 | 10.55 | 9.13 |
| BWG | 269.02 | 99.69 | 12561.64 | 7684.60 | 0.1342 | 0.0815 | 0.1985 | 13.14 | 7.65 |
| LBS | 273.94 | 130.83 | 23444.76 | 7642.55 | 0.1183 | 0.0069 | 0.2545 | 3.98 | 17.81 |
| PPC | 324.19 | 35.98 | 15148.06 | 13307.98 | 0.0367 | 0.0017 | 0.079 | 10.84 | 15.36 |
| SOG | 215.32 | 121.80 | 24079.30 | 10973.24 | 0.1079 | 0.0215 | 0.2135 | 15.47 | 12.07 |
| WPC | 163.16 | 81.28 | 14442.69 | 7020.62 | 0.0179 | 0.0016 | 0.0378 | 10.48 | 14.34 |
| WWG | 175.36 | 117.20 | 20974.15 | 6317.33 | 0.4928 | 0.2171 | 0.8298 | 18.24 | 6.34 |

*(from Zhang and Lamb 2011)

### *4.3.2 Statistical Analysis*

#### *4.3.2.1 Initial Experiment*

We developed and compared 12 *a priori* models each testing a theory linking community characteristics and productivity (Table 4.2; for more detail on the variables used in models see Appendix Table 6.5). We selected models that a) had a statistically significant variance explained relative to a null model, b) differentiated between statistically adequate models by comparing $R^2$ values, and c) potential utility of the model for predictive purposes (i.e. had explanatory variables that most easily linked to species composition). In each model, we compared the fit of a model with a linear relationship with the fit of a null model with only an intercept, using the "lm" function in the R package (Crawly 2007, R Development Core Team 2011). The response variable (productivity) was log transformed in all analyses.

**Table 4.2.** Summary of general linear models, rational for these a priori theories, and model fit for the models with data from the initial experiment. Highlighted models had the highest $R^2$ and were considered for the best model. Response variable is log (productivity (g)) for all models.

| Model | Explanatory Variables | Rational | AIC | F | p-value | $R^2$ |
|---|---|---|---|---|---|---|
| A | Species richness | Increase in productivity with increasing species richness as more traits become present and increased stability due to increased redundancy; (Lehman and Tilman 2000; Kirwan et al. 2007) | 27.537 | $F_{1,153}$=9.064 | 0.003 | 0.06 |
| B | Evenness | Increase in productivity with increasing evenness due to an increasing number of interactions (Kirwan et al. 2007) | 36.457 | $F_{1,153}$=0.005 | 0.944 | <0.01 |
| C | Functional group richness | Increase in productivity with increasing functional group richness as more traits become present and increased stability due to increased redundancy at a more general scope than species richness (Walker 1995; Reich et al. 2004; Balvanera et al. 2006) | 27.526 | $F_{1,153}$=9.075 | 0.003 | 0.06 |
| D | The abundance of each species | Increase in productivity with increased abundance of species suited to environmental conditions. (Tilman et al. 1996; Hooper et al. 2005) | 22.084 | $F_{7,147}$=3.898 | <0.001 | 0.16 |
| E | The abundance of each functional group | Increase in productivity with increased abundance of functional groups suited to environmental conditions. (Hooper et al. 2005) | 21.901 | $F_{3,151}$=6.401 | <0.001 | 0.11 |
| F | The presence of each species | Increase in productivity with the presence of species in the community due to high complimentarity or facilitation of that species to the other plants. (Hooper et al. 2005; Johnson et al. 2008) | 27.190 | $F_{7,147}$=3.088 | 0.005 | 0.13 |

| Model | Explanatory Variables | Rational | AIC | F | p-value | $R^2$ |
|---|---|---|---|---|---|---|
| G | The occurrence of each functional group | Increase in productivity with the presence of functional groups in the community due to high complimentarity or facilitation of that functional group to the other plants. (Hooper et al. 2005) | 11.008 | $F_{3,151}$=10.530 | <0.001 | 0.17 |
| H | Total relative growth rate | Increase in productivity as a result of high relative growth rate. (Levang-Brilz and Biondini 2003) | 19.164 | $F_{1,153}$=18.060 | <0.001 | 0.11 |
| I | Early (day 0-55) and late (day 55-100) relative growth rate | Increase in productivity as a result of high early relative growth rate or late relative growth rate. (Levang-Brilz and Biondini 2003) | 17.320 | $F_{2,152}$=11.100 | <0.001 | 0.13 |
| J | Specific leaf area (SLA) at two time points (Early: day 55, and Late: Day 100) | Increased productivity corresponds to higher SLA as a result of reduced investment into leaf photosynthates (Grime 1977; Craine et al. 2002; Diaz et al. 2004; Kembel and Cahill 2011) | 36.276 | $F_{2,152}$=1.077 | 0.343 | 0.01 |
| K | Specific root length at two time points (Early: day 55, and Late: Day 100) | Increases productivity in response to increases in SRL in response to competition (Aerts et al. 1991; Craine et al. 2002; Diaz et al. 2004; Weigelt et al. 2005; Kembel and Cahill 2011) | 36.182 | $F_{2,152}$=1.124 | 0.328 | 0.01 |
| L | Competitive response and effect | Increases in productivity as a result of high competitive response, and low competitive effect (Goldberg et al. 1990; Goldberg 1996; Wang et al. 2010; Zhang and Lamb 2011) | 16.134 | $F_{2,152}$=11.770 | <0.001 | 0.13 |

.*see Appendix Table 6.5 for more detail on the variables used in the models

#### *4.3.2.2 Confirmatory Experiment*

We tested if the equation from model D accurately predicted productivity using a general linear model where 'predicted productivity' was the explanatory variable and 'observed productivity' was the response variable. We compared the fit a model with a linear relationship to the fit of a null model with only an intercept, using the "lm" function in the R package (R Development Core Team 2011).

#### *4.3.2.3 Within Community Variation Experiment*

We estimated the average coefficient of variation across the six replicated communities. To assess if the communities predicted to have low productivity had lower productivity than those predicted to have high productivity, we performed an analysis of variance where the explanatory variable was a categorical variable with either low or high 'predicted productivity', and the response variable was 'observed productivity', using the "aov" function in the R package (R Development Core Team 2011).

## *4.4* Results

### *4.4.1 Drivers of community productivity*

Species richness, functional group richness, species and functional group abundance and occurrence, relative growth rate (RGR), and competitive ability were all significant drivers of productivity in the initial experiment as models had adequate fit (Table 4.2). Specifically, increases in species richness, functional group richness, the presence of WWG and $C_3$ grasses, early RGR, and total RGR, and decreases in competitive effect all significantly increased productivity (Table 4.3).

**Table 4.3.** Summary of direct effects for the models with data from the initial experiment. B, nodding brome, *Bromus anomalus*; BWG, blue bunch wheatgrass, *Pseudoregneria spicata*; LBS, little blue stem, *Schizachyrium scoparium*; PPC, purple prairie clover, *Dalea purpurea*; SOG, side oats grama, *Bouteloua curtipendula*; WPC, white prairie clover, *Dalea candida*; WWG, western wheatgrass, *Pascopyrum smithii*; $C_3$, cool season grass; $C_4$, warm season grass.

| Model | Parameter | Estimate | SE | t-value | p-value |
|---|---|---|---|---|---|
| A | Species richness | 0.0383 | 0.013 | 3.011 | 0.003 |
| B | Species evenness | -0.0126 | 0.178 | -0.071 | 0.944 |
| C | Functional group richness | 0.1059 | 0.035 | 3.012 | 0.003 |
| D | B | 0.0058 | 0.017 | 0.332 | 0.741 |
| | BWG | 0.0057 | 0.017 | 0.329 | 0.743 |
| | LBS | -0.0173 | 0.017 | -1.047 | 0.297 |
| | PPC | -0.0040 | 0.017 | -0.242 | 0.810 |
| | SOG | -0.0039 | 0.017 | -0.224 | 0.823 |
| | WPC | -0.0137 | 0.017 | -0.814 | 0.417 |
| | WWG | 0.0159 | 0.017 | 0.967 | 0.335 |
| E | legume | -0.0104 | 0.016 | -0.640 | 0.523 |
| | $C_3$ | 0.0074 | 0.016 | 0.456 | 0.649 |
| | $C_4$ | -0.0115 | 0.017 | -0.695 | 0.488 |
| F | B | 0.0522 | 0.045 | 1.167 | 0.245 |
| | BWG | 0.0814 | 0.044 | 1.839 | 0.068 |
| | LBS | -0.0118 | 0.046 | -0.257 | 0.798 |
| | PPC | 0.0057 | 0.044 | 0.129 | 0.897 |
| | SOG | 0.0482 | 0.043 | 1.132 | 0.260 |
| | WPC | -0.0691 | 0.046 | -1.499 | 0.136 |
| | WWG | 0.1363 | 0.046 | 2.944 | 0.004 |
| G | legume | -0.0174 | 0.060 | -0.289 | 0.773 |
| | $C_3$ | 0.3771 | 0.068 | 5.530 | <0.001 |
| | $C_4$ | -0.0086 | 0.060 | -0.143 | 0.887 |
| H | Total relative growth rate | 0.9291 | 0.219 | 4.249 | <0.001 |
| I | Early relative growth rate | 2.5063 | 1.028 | 2.438 | 0.016 |
| | Late relative growth rate | -0.0108 | 0.240 | -0.045 | 0.964 |

| Model | Parameter | Estimate | SE | t-value | p-value |
|---|---|---|---|---|---|
| J | Early specific leaf area | -0.0004 | 0.001 | -0.439 | 0.662 |
| | Late specific leaf area | 0.0012 | 0.001 | 1.128 | 0.261 |
| K | Early specific root length | 0.0000 | 0.000 | 1.446 | 0.150 |
| | Late specific root length | 0.0000 | 0.000 | -0.850 | 0.397 |
| L | Competitive response | 0.0037 | 0.015 | 0.253 | 0.801 |
| | Competitive effect | -0.0488 | 0.016 | -3.028 | 0.003 |

The models with the highest $R^2$ were model D where the abundance of each species drove productivity and model G where the occurrence of functional groups drove productivity (Table 4.3). As model D provided a more specific description of the community (species abundances rather than functional group occurrence) than model G, we used this model to select communities for the confirmatory experiment as described above. However, within this model, none of the coefficients were significant due to inter-correlation of the variables. This is an artifact of the experimental design because the pots were constrained to 21 plants; thus the abundance of a given species in the pot is constrained by the abundance of the other species. The equation from model D validated the experimental approach as a predictive tool because it accurately predicted productivity in the confirmatory experiment (Figure 4.1).

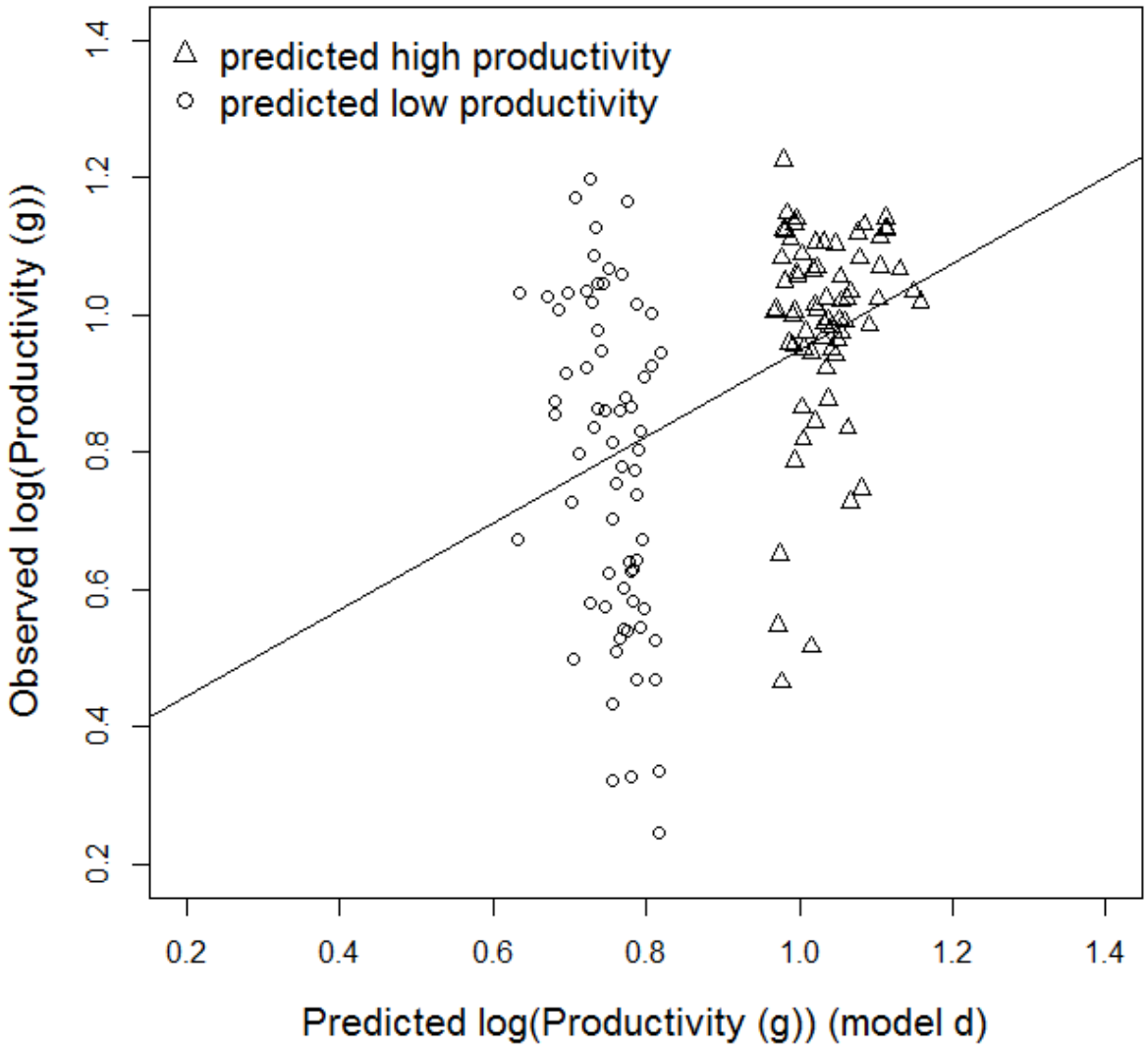


**Figure 4.1.** Regression of observed log productivity from the confirmatory experiment vs. predicted productivity derived from the equation from model D fit with data from the initial experiment ($F_{1,\ 136}$=28.64, p<0.0001, $R^2$ = 0.1739, y = (0.63035) x + 0.90328).

*4.4.2 Variation in community productivity*

Variation around the mean varied greatly between communities with an average coefficient of variation of 39% (Figure 4.2). However despite this variation, communities predicted to have

high productivity trended towards having significantly higher productivity than the communities predicted to have low productivity. The high variation within communities predicted to have low productivity is likely responsible for the low $R^2$s in the models reported in this paper.

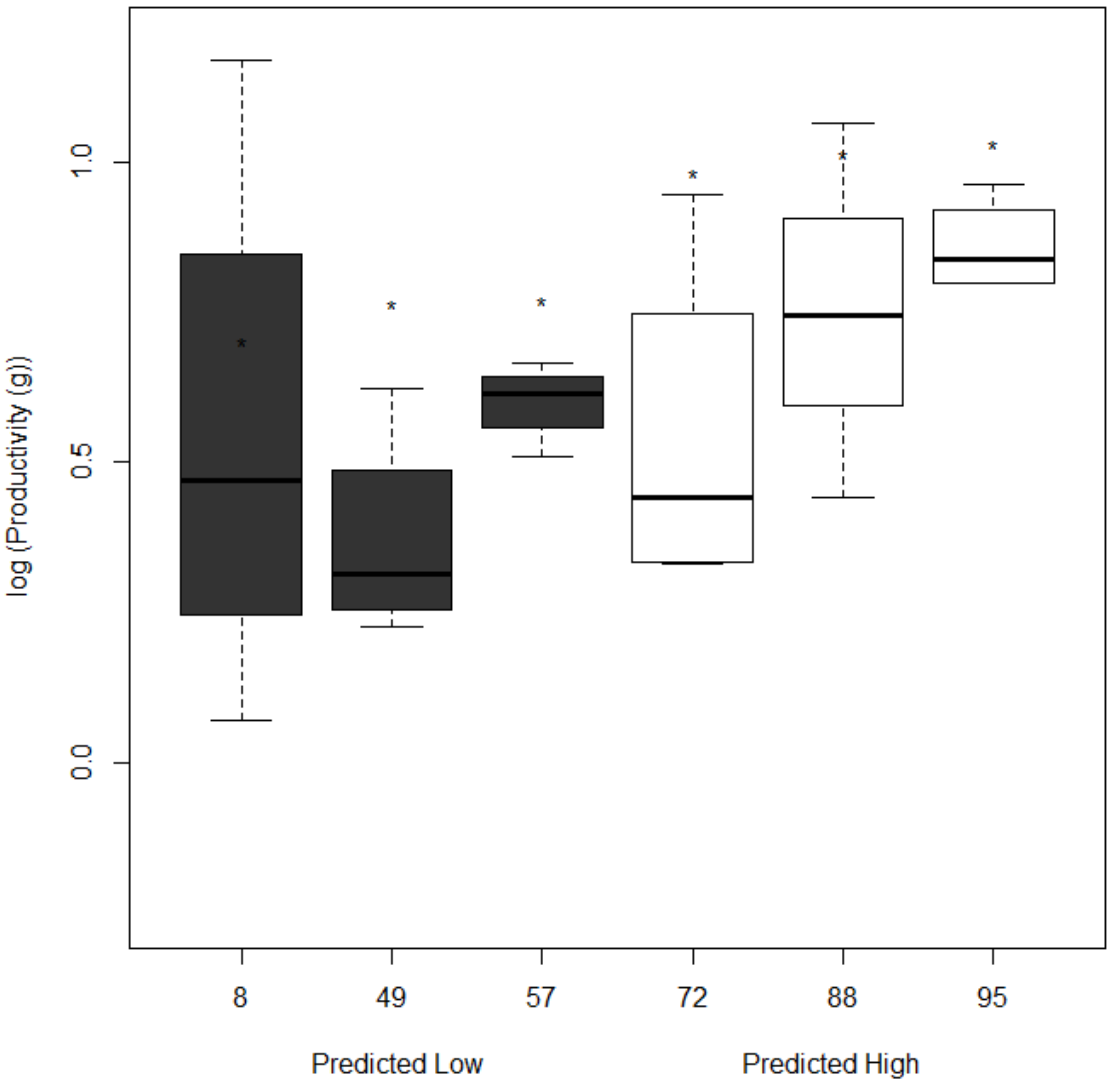


**Figure 4.2.** Variation of productivity for three communities predicted to have low productivity and three communities predicted to have high productivity. Numbers (8, 49, 57, 72, 88, 95) are community identity labels and are ranked by predicted productivity. The thick horizontal line is the median, the lower and upper bounds of the box represent the 25th and 75th percentiles respectively, the whiskers represent the 95% quartiles. * indicate predicted productivity. Productivity trended towards significantly different between communities predicted to have low productivity and communities predicted to have high productivity ($F_{1,21}$=3.433, p=0.078).

## 4.5 Discussion

We proposed and tested a method for assembling species and community compositions when there is no natural template to guide mixture design. Using this approach, communities of native or agronomic species can be tailored to optimize productivity. We validated the experimental approach and predictive tool in a confirmatory experiment where the communities predicted to have high productivity had high productivity. We further confirmed that community features including increased species richness, functional group richness, the presence and abundance of western wheatgrass and other $C_3$ grasses, average relative growth rate, and decreases in competitive effect ability all significantly increased productivity. Furthermore, the abundance of individual species can be used to successfully predict high productivity.

Despite variation within communities, underlying community signals driving high productivity were captured. Increased diversity may lead to increased productivity due to complementarity between plants, where increases in species and functional group richness resulted in overyielding (Trenbath 1974; Hooper et al. 2005). These results relate strongly to western wheatgrass, however, this may be due, in part, to a selection effect where higher species richness and functional group richness increase the probability that WWG is included in the community. Wheatgrass species have been shown to be initially successful after disturbance when available nitrogen is higher (Hammermeister et al. 2003). The high RGR of WWG impacts the community by contributing biomass, and structuring the community through increased litter and soil stabilization, but through competition may reduce availability of resources to other species (Grime and Hunt 1975). Furthermore, the strong effect of early RGR indicates that this early season growth is more influential on the short-term productivity of the community than later RGRs. Aggressive, competitive species with high RGR and large size (correlated with a

high competitive effect) may increase productivity initially; however, plants that grow more slowly with higher investment in root and leaf tissue may have increased tolerance for environmental stress (Grime and Hunt 1975; Wang et al. 2010). Not all traits significantly predicted productivity in the experiment; SLA and SRL may not have influenced the productivity these communities because the range of SLA among these given species was not large enough to drive a change in productivity (Diaz et al. 2004). Similarly, the effect of SRL may have been reduced by the limited rooting depth in the pots (Aerts et al. 1991).

We expected both legumes and $C_4$ grasses to contribute to community productivity; however the short-term nature of the experiment may have obscured those contributions. Legumes can be useful in mixtures as they can increase available nitrogen and reduce fertilizer requirements in nitrogen deficient rangeland systems (Callaway 1995; Brooker et al. 2008; Whitbread et al. 2009; Muir et al. 2011). This was not evident in our study, likely due to the low RGR of the legumes and limited time for nitrogen recruitment from legume litter. $C_4$ grasses were expected to contribute to productivity in part due to their ability to withstand warmer temperatures and lower moisture. Greenhouse conditions were likely better suited to $C_3$ grass species as the average greenhouse temperature was 23°C and only rarely reached the maximum of 36°C. Therefore, experimenting with environmental conditions in a more controlled environment, such as a growth chamber, may result in an increase in the importance of including $C_4$ grasses in the community. In this study, experiments were terminated after a full lifecycle of the plants and not continued due to pot constraints. This better reflects a short term lifespan, thus the experimental approach may be better suited to screening annual species in the greenhouse. To achieve results that reflect mature stand productivity of perennial communities, the approach could be applied using field trials but long term assessment would be required.

There was large variation in productivity between pots evident in both the initial and confirmatory experiments (low $R^2$ values for models), and within the same communities in the repetition experiment. This variation likely arises from a number of sources, particularly changing interaction dynamics driven by the spatial location of plants within pots. Additional sources of noise may include the genetic variation and phenotypic plasticity within the species included in the experiment. Volin et al. (1998) for example, reported very different absolute values for the RGR and SLA of WWG, LBS, and SOG than this study, but the rank order of the trait values was consistent between the two studies. Many replicates would be required to precisely evaluate the productivity of any individual community, something unfeasible given nearly 300 000 possible community configurations. The power of the response surface design used here is in the ability to screen multiple large numbers of community configurations to identify the community characteristics associated with high productivity on average. Despite the noisy data, the trends driving community productivity were clearly evident in these results, and the predictive equations successfully identified high productivity communities in the confirmation experiment.

## 4.6 Conclusions

Our screening method successfully predicted communities with high potential productivity. Increases in diversity, and the inclusion of aggressive, competitive species significantly increased productivity. The best models utilized the abundance of each species, and the occurrence of functional groups. The experimental approach and predictive tool developed were validated in a confirmatory experiment where the communities predicted to have high productivity had high productivity. This approach could be used as a general tool to screen

species and potential community compositions when designing seed mixtures for ecological restoration.

## 4.7 References


**Aerts, R., Boot, R. G. A. and van der Aart, P. J. M. 1991**. The relation between above- and belowground biomass allocation patterns and competitive ability. Oecologia. **87**: 551-559.

**Balvanera, P., Pfisterer, A. B., Buchmann, N., He, J. S., Nakashizuka, T., Raffaelli, D. and Schmid, B. 2006**. Quantifying the evidence for biodiversity effects on ecosystem functioning and services. Ecol. Lett. **9**: 1146-1156.

**Bertness, M. D. and Callaway, R. 1994**. Positive interactions in communities. Trends Ecol. Evol. **9**: 191-193.

**Brooker, R. W., Maestre, F. T., Callaway, R. M., Lortie, C. L., Cavieres, L. A., Kunstler, G., Liancourt, P., Tielbörger, K., Travis, J. M. J., Anthelme, F., Armas, C., Coll, L., Corcket, E., Delzon, S., Forey, E., Kikvidze, Z., Olofsson, J., Pugnaire, F., Quiroz, C. L., Saccone, P., Schiffers, K., Seifan, M., Touzard, B. and Michalet, R. 2008**. Facilitation in plant communities: the past, the present, and the future. J. Ecol. **96**: 18-34.

**Bruno, J. F., Stachowicz, J. J. and Bertness, M. D. 2003**. Inclusion of facilitation into ecological theory. Trends Ecol. Evol. **18**: 119-125.

**Cahill, J. F. 2003**. Lack of relationship between below-ground competition and allocation to roots in 10 grassland species. J. Ecol. **91**: 532-540.

**Callaway, R. M. 1995**. Positive interactions among plants. Bot. Rev. **61**: 306-349.

**Callaway, R. M. 2007**. Positive interactions and interdependence in plant communities. Springer. Pp 415.

**Choler, P., Michalet, R. and Callaway, R. M. 2001**. Facilitation and competition on gradients in alpine plant communities. Ecology. **82**: 3295-3308.

**Cornelissen, J. H. C., Lavorel, S., Garnier, E., Díaz, S., Buchmann, N., Gurvich, D. E., Reich, P. B., Steege, H. t., Morgan, H. D., Heijden, M. G. A. v. d., Pausas, J. G. and Poorter, H. 2003**. A handbook of protocols for standardised and easy measurement of plant functional traits worldwide. Aust. J. Bot. **51**: 335-380.

**Craine, J. M., Tilman, D., Wedin, D., Reich, P., Tjoelker, M. and Knops, J. 2002**. Functional traits, productivity and effects on nitrogen cycling of 33 grassland species. Funct. Ecol. **16**: 563-574.

**Crawley, M. J. 2007**. The R Book. John Wiley & Sons, Chichester, U. K.

**Díaz, S., Hodgson, J. G., Thompson, K., Cabido, M., Cornelissen, J. H. C., Jalili, A., Montserrat-Martí, G., Grime, J. P., Zarrinkamar, F., Asri, Y., Band, S. R., Basconcelo, S., Castro-Díez, P., Funes, G., Hamzehee, B., Khoshnevi, M., Pérez-Harguindeguy, N., Pérez-Rontomé, M. C., Shirvany, F. A., Vendramini, F., Yazdani, S., Abbas-Azimi, R., Bogaard, A., Boustani, S., Charles, M., Dehghan, M., de Torres-Espuny, L., Falczuk, V., Guerrero-Campo, J., Hynd, A., Jones, G., Kowsary, E., Kazemi-Saeed, F., Maestro-Martínez, M., Romo-Díez, A., Shaw, S., Siavash, B.,**

**Villar-Salvador, P. and Zak, M. R. 2004**. The plant traits that drive ecosystems: Evidence from three continents. J. Veg. Sci. **15**: 295-304.

**Goldberg, D. E. 1996**. Competitive ability: Definitions, contingency and correlated traits. Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences. **351**: 1377-1385.

**Goldberg, D. E., Grace, J. and Tilman, D. 1990**. Components of resource competition in plant communities. Perspectives on plant competition. Pp 27-49.

**Grime, J. P. 1974**. Vegetation classification by reference to strategies. Nature. **250**: 26-31.

**Grime, J. P. 1977**. Evidence for the existence of three primary strategies in plants and its relevance to ecological and evolutionary theory. The American Society of Naturalists. **111**: 1169-1194.

**Grime, J. P. and Hunt, R. 1975**. Relative growth-rate: Its range and adaptive significance in a local flora. J. Ecol. **63**: 393-422.

**Hammermeister, A. M., Naeth, M. A., Schoenau, J. J. and Biederbeck, V. O. 2003**. Soil and plant response to wellsite rehabilitation on native prairie in southeastern Alberta, Canada. Can. J. Soil Sci. **83**: 507-519.

**Hooper, D. U., Chapin, F. S., Ewel, J. J., Hector, A., Inchausti, P., Lavorel, S., Lawton, J. H., Lodge, D. M., Loreau, M., Naeem, S., Schmid, B., Setala, H., Symstad, A. J., Vandermeer, J. and Wardle, D. A. 2005a**. Effects of biodiversity on ecosystem functioning: A consensus of current knowledge. Ecol. Monogr. **75**: 3-35.

**Jefferson, P. G., Iwaasa, A. D., Schellenberg, M. P. and McLeod, J. G. 2005**. Re-evaluation of native plant species for seeding and grazing by livestock on the semiarid prairie of western Canada. Prairie Forum. **30**: 85-106.

**Johnson, D., Phoenix, G. K. and Grime, J. P. 2008**. Plant community composition, not diversity, regulates soil respiration in grasslands. Biology Letters. **4**: 345-348.

**Kembel, S. W. and Cahill, J. F., Jr. 2011**. Independent Evolution of Leaf and Root Traits within and among Temperate Grassland Plant Communities. PLoS ONE. **6**: e19992.

**Kirwan, L., Luescher, A., Sebastia, M. T., Finn, J. A., Collins, R. P., Porqueddu, C., Helgadottir, A., Baadshaug, O. H., Brophy, C., Coran, C., Dalmannsdottir, S., Delgado, I., Elgersma, A., Fothergill, M., Frankow-Lindberg, B. E., Golinski, P., Grieu, P., Gustavsson, A. M., Hoglind, M., Huguenin-Elie, O., Iliadis, C., Jorgensen, M., Kadziuliene, Z., Karyotis, T., Lunnan, T., Malengier, M., Maltoni, S., Meyer, V., Nyfeler, D., Nykanen-Kurki, P., Parente, J., Smit, H. J., Thumm, U. and Connolly, J. 2007**. Evenness drives consistent diversity effects in intensive grassland systems across 28 European sites. J. Ecol. **95**: 530-539.

**Lamb, E., Kennedy, N. and Siciliano, S. 2011**. Effects of plant species richness and evenness on soil microbial community diversity and function. Plant Soil. **338**: 483-495.

**Lehman, Clarence L. and Tilman, D. 2000**. Biodiversity, stability, and productivity in competitive communities. Am. Nat. **156**: 534-552.

**Levang-Brilz, N. and Biondini, M. E. 2003**. Growth rate, root development and nutrient uptake of 55 plant species from the Great Plains Grasslands, USA. Plant Ecol. **165**: 117-144.

**Loreau, M., Naeem, S., Inchausti, P., Bengtsson, J., Grime, J. P., Hector, A., Hooper, D. U., Huston, M. A., Raffaelli, D., Schmid, B., Tilman, D. and Wardle, D. A. 2001**. Ecology - Biodiversity and ecosystem functioning: Current knowledge and future challenges. Science. **294**: 804-808.

**Maestre, F. T., Valladares, F. and Reynolds, J. F. 2005**. Is the change of plant–plant interactions with abiotic stress predictable? A meta-analysis of field results in arid environments. J. Ecol. **93**: 748-757.

**Miller, T. E. 1994**. Direct and indirect species interactions in an early old-field plant community. The American Society of Naturalists. **143**: 1007-1025.

**Mischkolz, J., Schellenberg, M. P. and Lamb, E. G. In press**. Early productivity and crude protein content of establishing forage swards composed of combinations of native grass and legume species in mixed-grassland ecoregions. Can. J. Plant Sci.

**Muir, J. P., Pitman, W. D. and Foster, J. L. 2011**. Sustainable, low-input, warm-season, grass-legume grassland mixtures: mission (nearly) impossible? Grass Forage Sci. **66**: 301-315.

**Prairie Conservation Action Plan Partnership. 2005**. Saskatchewan prairie conservation action plan 2003-2008. Pp 46.

**Reich, P. B., Tilman, D., Naeem, S., Ellsworth, D. S., Knops, J., Craine, J., Wedin, D. and Trost, J. 2004**. Species and functional group diversity independently influence biomass accumulation and its response to CO2 and N. Proc. Natl. Acad. Sci. U. S. A. **101**: 10101-10106.

**Schellenberg, M. P. and Banerjee, M. R. 2002**. The potential of legume-shrub mixtures for optimum forage production in southwestern Saskatchewan: A greenhouse study. Can. J. of Plant Sci. **82**: 357-363.

**Sheehan, C., Kirwan, L., Connolly, J. and Bolger, T. 2006**. The effects of earthworm functional group diversity on nitrogen dynamics in soils. Soil Biology and Biochemistry. **38**: 2629-2636.

**Smith, B. and Wilson, J. B. 1996**. A consumer's guide to evenness indices. Oikos. **76**: 70-82.

**Symstad, A. J., Chapin, F. S., Wall, D. H., Gross, K. L., Huenneke, L. F., Mittelbach, G. G., Peters, D. P. C. and Tilman, D. 2003**. Long-term and large-scale perspectives on the relationship between biodiversity and ecosystem functioning. Bioscience. **53**: 89-98.

**Tilman, D. 1997**. Community invasibility, recruitment limitation, and grassland biodiversity. Ecology. **78**: 81-92.

**Tilman, D., Wedin, D. and Knops, J. 1996**. Productivity and sustainability influenced by biodiversity in grassland ecosystems. Nature. **379**: 718-720.

**Trenbath, B. R. 1974**. Biomass productivity of mixtures. Adv. Agron. **26**: 177-210.

**Volin, J. C., Reich, P. B. and Givnish, T. J. 1998**. Elevated carbon dioxide ameliorates the effects of ozone on photosynthesis and growth: Species respond similarly regardless of photosynthetic pathway or plant functional group. New Phytol. **138**: 315-325.

**Walker, B. 1995**. Conserving Biological Diversity through Ecosystem Resilience
La conservación de la diversidad biológica a través de la resiliencia de los ecosistemas. Conserv. Biol. **9**: 747-752.

**Wang, P., Stieglitz, T., Zhou, D. W. and Cahill Jr, J. F. 2010**. Are competitive effect and response two sides of the same coin, or fundamentally different? Funct. Ecol. **24**: 196-207.

**Weigelt, A., Steinlein, T. and Beyschlag, W. 2005**. Competition among three dune species: the impact of water availability on below-ground processes. Plant Ecol. **176**: 57-68.

**Weigelt, A., Weisser, W. W., Buchmann, N. and Scherer-Lorenzen, M. 2009**. Biodiversity for multifunctional grasslands: equal productivity in high-diversity low-input and low-diversity high-input systems. Biogeosciences. **6**: 1695-1706.

**Whitbread, A. M., Hall, C. A. and Pengelly, B. C. 2009**. A novel approach to planting grass-legume pastures in the mixed farming zone of southern inland Queensland, Australia. Crop Pasture Sci. **60**: 1147-1155.

**Zhang, S. and Lamb, E. G. 2011**. Plant competitive ability and the transitivity of competitive hierarchies change with plant age. Plant Ecol. **213**: 15-23.

# 5 GENERAL CONCLUSIONS

Seed of species native to the Canadian Mixed Grass Prairie is commercially available, but there is little information on how to combine species into effective multispecies forage swards for prairie restoration and seeded pastures. Commercial seed mixture development has relied on experience and personal judgments rather than a more methodological approach to selecting species due to limited knowledge on the features of species mixtures that provide high and consistent forage production. The focus of this thesis was to determine if complex mixtures of these species could provide a sustainable, drought tolerant, non-invasive, productive rangeland for pasture use in the Mixed Grassland; and to design a systematic way to identify the best mixtures. We worked here with seven native species in the Mixed Grass Prairie ecoregion. We first investigated the early productivity and nutritional quality of simple mixtures of the selected native species in a field study by establishing and harvesting forage swards composed of monocultures and pairs of species to assess their productivity and crude protein. We developed and tested a new approach to assemble complex seed mixtures in greenhouse experiments through identification of the community characteristics that are the strongest predictors of high productivity. Finally, we tested if the screening tool and the mixtures identified thereby were robust to a moderate drought.

Results were largely consistent between the field (Chapter 2) and greenhouse studies (Chapters 3 and 4). Among the species tested, western wheatgrass (WWG) had the highest overall plant density and the strongest effect on the forage yield of the forage swards and communities. WWG has extremely high potential as a perennial forage sward, as it is easily established and grows into a dense stand with sod-forming rhizomes (National Research Council

2000; United States Department of Agriculture National Resources Conservation Service Plant Materials Program 2002). As the only sod-forming $C_3$ in this study it may represent a more competitive life strategy, possibly due to high early relative growth rate (Chapter 4) and colonization potential (Monsen et al. 2004). This likely contributed to WWG being the most productive species in the early establishment of these forage swards, especially as drought conditions did not limit WWG (Wang and Schellenberg 2012). Wheatgrasses have been shown to be successful initially after disturbance where available nitrogen is higher and WWG is a competitive dominant as both a seedling and mature plant (Zhang and Lamb 2011). In the field study (Chapter 2), productivity and crude protein content were not reduced when other species were also included with WWG in the forage sward, despite WWG being seeded at a 50% lower rate. Thus, inclusion of less productive species in the forage sward carried little penalty for pasture productivity or nutritional value. Less productive species thus should be included in pasture mixes when they bring beneficial traits (i.e. higher tolerance to grazing, drought resistance) to the restored forage sward. Including these traits carries little penalty under good growing conditions, and provides "insurance" for less optimal years.

*Dalea* spp. did not establish as well as the other species in the field (Chapter 2), but had the highest crude protein concentrations. The legumes had lower plant densities than the grasses, which reduced their effects on the forage sward. Legume abundance may increase with moderate grazing, where forbs increase as grasses decrease (Bai et al. 2001). Purple prairie clover is known to have high protein, low fibre content, and is rated as a fair forage sward that increases under moderate grazing but suffers when overgrazed (Schellenberg and Banerjee 2002; McGraw et al. 2004; Tannas 2004). Unpublished data indicates prairie clovers require higher temperatures than grasses to initiate germination (Schellenberg and Henderson 2010), and are slow to establish

with relatively low forage yield (Schellenberg and Banerjee 2002; Molano-Flores et al. 2011). Legume impacts on soil nitrogen were not detectable within the first two years of being seeded, however as the clovers become more established the benefits of including legumes may become evident as more nitrogen is fixed into the soil.

In our greenhouse study (Chapter 4), 12 *a priori* theories were used to successfully predict high community productivity. The best model was used as a predictive tool to screen other potential mixtures. Productivity increased with increases in all aspects of diversity including species richness and functional group richness as in Lehman and Tilman (2000). One of the largest drivers of productivity was the presence of $C_3$ grasses, particularly WWG. The strongest predictors of productivity were the presence and abundance of perennial $C_3$ grasses. Facilitation was evident as many of the communities had higher productivity than others; positive interactions within these communities may be more evident as the plant mature. We captured the best predictor of high productivity for the establishment phase of these communities, although there was large variation in productivity between pots evident in both the initial and confirmatory experiments (low $R^2$ values for models), and within the same communities in the repetition experiment (Chapter 4). This variation may be in part due to the changing interaction dynamics driven by the spatial location of plants within pots. Further understanding of these interactions may be fruitful to identify plant specific responses to their neighbours and better understand how they are related to the community's total productivity. For example, little bluestem was negatively affected by other species (including side oats grama) in a study by Weatherford and Myster (2011). Therefore, perhaps the lack of success of these $C_4$ species in this study is due to niche overlap and strong competition when planted as neighbours, which would not be evident without incorporating this spatial information.

An alternative approach for assembling species and community compositions when there is no natural template was proposed. This approach allows for communities to be tested and tailored for any particular region. The experimental approach and predictive tool developed were validated in a confirmatory experiment where the communities predicted to have high productivity had high productivity. The abundance of individual species was successfully used to predict high productivity. Applied restoration ecologists may find this approach useful when considering which species, and in what proportion, to seed grasslands; furthermore, it can be modified to consider enhancing other ecological functions in addition to high productivity.

More diverse forage swards may provide a more reliable source of forage yield even in years with very different environmental conditions (Lehman and Tilman 2000; Loreau and de Mazancourt 2013). In our field study (Chapter 2), species that benefited from the wet year ($C_3$ grasses) and rapidly established (WWG) were highly productive; however, in a more typical year, drought tolerant species may compensate for the $C_3$ grasses which become dormant in hot, dry conditions. The effects of drought in the greenhouse experiment were not strong (Chapter 3), possibly because these species are native to the semi-arid grassland and thus adapted to water limited conditions (Turner 1986). Productivity was, however, affected by the moderate drought to some degree but the relative importance of community characteristics driving productivity did not change. As these species all seem to tolerate moderate drought, drought may not be necessary to consider when screening communities of these species.

In both the field and greenhouse settings, the biomass production during early establishment phase of these communities was dominated by fast growing and highly competitive species. The overlap in results is reassuring as greenhouse experiments allow increased control designed to tease out more specific relationships, as well as more mixtures to

be tested, although it does not contain the complexity of the natural environment. During this study, precipitation was unusually high resulting in cool moist soil in the field study (Chapter 2), and the greenhouse conditions may not have been warm enough to generate a strong dormancy in the $C_3$ grasses (Chapters 3 and 4). As dormancy limits the growth of $C_3$ species, the cool moist environmental conditions may explain the success of the $C_3$ grasses compared to the $C_4$ grasses.

All results within the time frame of this study are during an early stage in these perennial species' lives, and are affected by establishment success. This is not surprising as some species may require more than two years to become established (Jefferson et al. 2002). Therefore, these results must be considered within the context of this early establishment phase. Further assessment of these forage swards over the long term is recommended to identify if these community dynamics change over time, with respective changes in community productivity.

This thesis's overall goal was to provide knowledge on how to compose suitable species mixtures. There is interest in seed mixtures for the Mixed Grass Prairie ecoregion that are sustainable, drought tolerant, low input, and non-invasive, thus native, perennial species with high agronomic potential (reasonable plant size, plant nutritional quality, cost, availability, and the ability to work with conventional machinery) were chosen. The early forage and crude protein yields of the selected species with simple community compositions were assessed in field plots (Chapter 2). The influence of water limited conditions on the selecting the best model was assessed where, the relative productivities were not altered (Chapter 3). Therefore, optimum communities are not likely to be different under drought conditions. Complex mixtures of the selected species were evaluated in the greenhouse to test predictive models (Chapter 4). The best model was confirmed to successfully predict high productivity, thereby validating our systematic method of assembling species into optimum communities. Continued monitoring of the field

sites, and the field establishment of complex mixtures with high productivity will help to determine if applying the approaches will provide communities with stable high productivity in the long term.

## 5.1 References


**Bai, Y., Abouguendia, Z. and Redmann, R. E. 2001**. Relationship between plant species diversity and grassland condition. J. Range Manag. **54**: 177-183.

**Jefferson, P. G., McCaughey, W. P., May, K., Woosaree, J., MacFarlane, L. and Wright, S. M. 2002**. Performance of American native grass cultivars in the Canadian prairie provinces. Native Plants Journal. **3**: 24-33.

**Lehman, C. L. and Tilman, D. 2000**. Biodiversity, stability, and productivity in competitive communities. Am. Nat. **156**: 534-552.

**Loreau, M. and de Mazancourt, C. 2013**. Biodiversity and ecosystem stability: a synthesis of underlying mechanisms. http://dx.doi.org/10.1111/ele.12073.

**McGraw, R. L., Shockley, F. W., Thompson, J. F. and Roberts, C. A. 2004**. Native legume species. Native Plants Journal. **5**: 152-159.

**Molano-Flores, B., Coons, J. M. and Cunningham, J. B. 2011**. Germination of two seed types from three *Dalea* species. Castanea. **76**: 266-271.

**Monsen, S. B., Stevens, R. and Shaw, N. 2004**. Chapter 18. Grasses. Pages 295-424 *in* Restoring western ranges and wildlands, vol. 2. USDA Forest Service Gen. Tech. Rep. RMRS-GTR-136.

**National Research Council. 2000**. Nutrient requirements of beef cattle. 7th rev. ed. 1996 (updated 2001). Subcommittee on Beef Cattle Nutrition, Committee on Animal Nutrition, Board on Agriculture, National Research Council. National Academic Press, Washington, DC. 381 pp.

**Schellenberg, M. P. and Banerjee, M. R. 2002**. The potential of legume-shrub mixtures for optimum forage production in southwestern Saskatchewan: A greenhouse study. Can. J. of Plant Sci. **82**: 357-363.

**Schellenberg, M. P. and Henderson, D. 2010**. Germination temperature characteristics of three Dalea spp. with overlapping ranges in the Canadian prairies. Abstract in: "Working landscapes, SRM and WSSA Joint AGM". Denver, CO. C-29.

**Tannas, K. 2004**. Common plants of the western rangelands: Forbs. Vol 3. Alberta Agriculture, Food and Rural Development. Pp 505.

**Turner, N. 1986**. Adaptation to water deficits: A changing perspective. Funct. Plant Biol. **13**: 175-190.

**United States Department of Agriculture National Resources Conservation Service Plant Materials Program. 2002**. Western Wheatgrass *Pascopyrum smithii* (Rydb.) Scribn. http://plants.usda.gov/java/profile?symbol=PASM. 31 July 2012. USDA Forest Service Gen. Tech. Rep. RMRS-GTR-136.

**Wang, Z. and Schellenberg, M. P. 2012**. Drought and N addition in a greenhouse experiment: blue grama and western wheatgrass. J. Agr. Sci. Tech. B. **2**: 29-37.

**Weatherford, J. L. and Myster, R. W. 2011**. Interactive effects of species, simulated grazing, and below-ground resources on competitive outcome among three prairie grasses. The Journal of the Torrey Botanical Society. **138**: 107-119.

**Zhang, S. and Lamb, E. G. 2011**. Plant competitive ability and the transitivity of competitive hierarchies change with plant age. Plant Ecol. **213**: 15-23.

# 6 GENERAL APPENDIX

## 6.1 Soil Moisture

An additional objective in this study was to assess if plant communities will affect the soil moisture level over the growing season, with $C_3$ grasses using more water. In Swift Current, we measured soil moisture to a depth of 15 cm every two weeks across the growing season with a portable Time Domain Reflectometry (TDR) probe (Trase Systems) in 2011 for two blocks.

We tested the effect of the forage swards on soil moisture across growing season using mixed models and the model selection approach described above, where the model included percent moisture as the response variable, forage sward as a fixed effect, and block and date as random effects. Soil moisture was affected by forage sward ($F_{4, 444}$=16.12, $p<0.0001$). SOGxPPC had the highest soil moisture, while BxWWG had the lowest soil moisture (Figure 6.1). The forage swards altered soil moisture content, which indicates differences in water use efficiencies among the species. $C_4$ grasses were included in part for their drought resistance which was not evident in such wet years. Benefits to including them may be more obvious in dryer years.

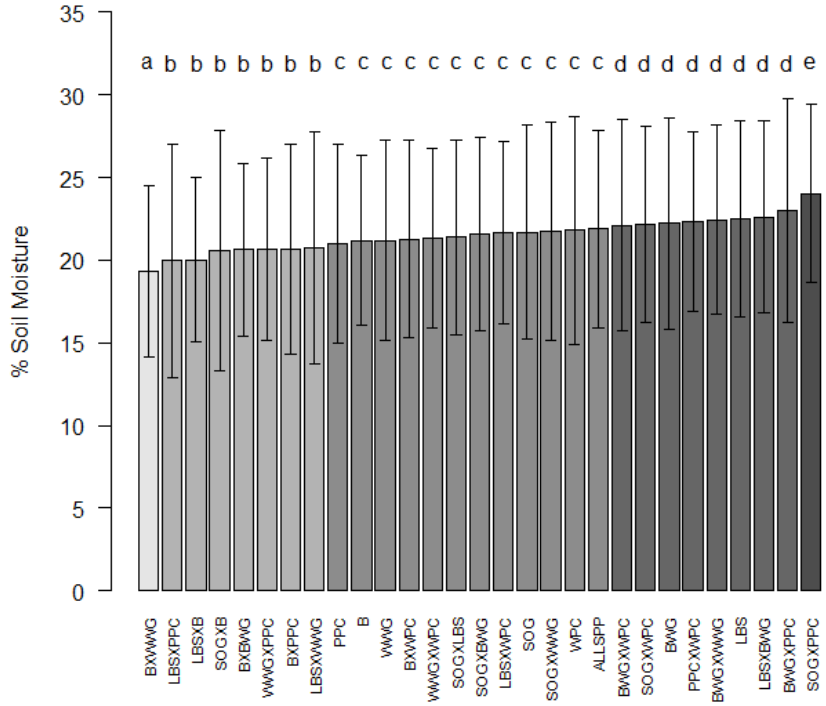


**Figure 6.1** Effect of the forage swards on soil moisture across sampling dates, ordered according to rank. Different letters indicate significant differences between forage swards. Error bars represent one standard deviation around the mean.

## 6.2 Trait Data Methods

To assess plant traits for each species, we followed the published guidelines for the assessment of leaf and root traits, and relative growth rate (RGR) measurements (Cornelissen et al. 2003; Levang-Brilz and Biondini 2003). Leaf and root traits were evaluated at time points: early (day 55), and late (day 100), and RGR was evaluated for three time periods: total (day 0-100), early (day 0-55), and late (day 55-100) (Tables 6.1-6.4). We planted eight seedlings of each species (1 plant/pot) and harvested four plants/species after 55 days (early sampling period), and four plants/species after 100 days (late sampling period). Plant death resulted in a minimum sample

size of three plants for each species, with additional plants when available. We scanned three mature leaves of each plant at a dpi of at least 400 for image analysis of one-sided projected leaf area using WinFOLIA software (Regent Instruments Inc., Sainte-Foy, Quebec, Canada), and measured the thickness of the lamina of each leaf to the nearest 0.1 mm using digital calipers. We extracted a subsample of the fine root system (defined as living roots with a diameter <2 mm) of each plant, after washing each plant over a sieve to remove soil. The root subsamples were stored in a 70% ethanol solution, and subsequently scanned at 800 dpi for image analysis of root length, volume, and average fine root diameter using WinRHIZO software (Regent Instruments Inc.). Both leaf and root subsamples were dried for 72 hours at 70 degrees Celsius, and weighed. In addition to direct measurement of leaf size (one sided projected leaf area;$cm^2$) and leaf thickness (mm) for each leaf, specific leaf area (SLA; leaf area per unit biomass; $cm^2/g$) and leaf tissue density (leaf biomass per unit volume; $mg/mm^3$; with leaf volume calculated as the product of leaf thickness and area) were estimated. Similarly, direct measures of fine root sample length, volume, and diameters were used to estimate specific root length (SRL; root length per unit mass; m/g) and root tissue density (root mass per unit volume; $mg/mm^3$) for each plant. After we took the subsamples from each plant for the trait measurements, the remaining material was split into above and below ground portions, dried for 72 hours at 70 degrees Celsius and weighed (subsample weights were added respectively to calculate total plant weight for above and below ground portions), and RGR was calculated as biomass/#days.

**Table 6.1**. Leaf measurements of three mature leaves from three plants of each species from raw data at age day 55, scanned at 600 dpi; all plants grown in greenhouse, destructively sampled. B, nodding brome, *Bromus anomalus*; BWG, blue bunch wheatgrass, *Pseudoregneria spicata*; LBS, little blue stem, *Schizachyrium scoparium*; PPC, purple prairie clover, *Dalea purpurea*; SOG, side oats grama, *Bouteloua curtipendula*; WPC, white prairie clover, *Dalea candida*; WWG, western wheatgrass, *Pascopyrum smithii*.

| Species | Plant | Leaf Number | Weight (g) | Thickness (mm) | Projected Leaf Area ($cm^2$) |
|---|---|---|---|---|---|
| b | 1 | a | 0.0291 | 6.604 | 8.5209 |
| b | 1 | b | 0.0349 | 5.842 | 6.5554 |
| b | 1 | c | 0.0257 | 9.144 | 6.6385 |
| b | 2 | a | 0.0427 | 7.62 | 9.0173 |
| b | 2 | b | 0.013 | 4.826 | 3.3751 |
| b | 2 | c | 0.03 | 6.858 | 5.958 |
| b | 3 | a | 0.0233 | 5.588 | 4.9617 |
| b | 3 | b | 0.0313 | 5.08 | 7.8998 |
| b | 3 | c | 0.0233 | 6.096 | 5.3548 |
| bwg | 1 | a | 0.0216 | 4.572 | 4.9788 |
| bwg | 1 | b | 0.0106 | 3.81 | 3.1717 |
| bwg | 1 | c | 0.0053 | 8.636 | 1.4413 |
| bwg | 2 | a | 0.0156 | 1.778 | 4.9352 |
| bwg | 2 | b | 0.011 | 3.556 | 3.7245 |
| bwg | 2 | c | 0.007 | 2.286 | 2.7878 |
| bwg | 3 | a | 0.0161 | 3.556 | 2.4873 |
| bwg | 3 | b | 0.0093 | 4.826 | 3.2317 |
| bwg | 3 | c | 0.0177 | 3.048 | 3.9637 |
| lbs | 1 | a | 0.0152 | 5.842 | 4.355 |
| lbs | 1 | b | 0.0101 | 6.858 | 2.2177 |
| lbs | 1 | c | 0.006 | 8.89 | 3.1991 |
| lbs | 2 | a | 0.0042 | 8.89 | 2.6839 |
| lbs | 2 | b | 0.0126 | 6.604 | 2.0621 |
| lbs | 2 | c | 0.0072 | 10.668 | 1.0062 |
| lbs | 3 | a | 0.003 | 6.096 | 3.6699 |
| lbs | 3 | b | 0.0122 | 6.604 | 3.1143 |
| lbs | 3 | c | 0.0147 | 7.62 | 1.0312 |
| ppc | 1 | a | 0.0011 | 11.938 | 0.5048 |
| ppc | 1 | b | 0.0014 | 9.398 | 0.4125 |
| ppc | 1 | c | 0.0019 | 10.922 | 0.5623 |
| ppc | 2 | a | 0.0013 | 8.128 | 0.428 |
| ppc | 2 | b | 0.0013 | 11.43 | 0.3682 |

| Species | Plant | Leaf Number | Weight (g) | Thickness (mm) | Projected Leaf Area ($cm^2$) |
|---|---|---|---|---|---|
| b | 1 | a | 0.0291 | 6.604 | 8.5209 |
| b | 1 | b | 0.0349 | 5.842 | 6.5554 |
| b | 1 | c | 0.0257 | 9.144 | 6.6385 |
| b | 2 | a | 0.0427 | 7.62 | 9.0173 |
| b | 2 | b | 0.013 | 4.826 | 3.3751 |
| b | 2 | c | 0.03 | 6.858 | 5.958 |
| b | 3 | a | 0.0233 | 5.588 | 4.9617 |
| b | 3 | b | 0.0313 | 5.08 | 7.8998 |
| b | 3 | c | 0.0233 | 6.096 | 5.3548 |
| bwg | 1 | a | 0.0216 | 4.572 | 4.9788 |
| bwg | 1 | b | 0.0106 | 3.81 | 3.1717 |
| bwg | 1 | c | 0.0053 | 8.636 | 1.4413 |
| bwg | 2 | a | 0.0156 | 1.778 | 4.9352 |
| bwg | 2 | b | 0.011 | 3.556 | 3.7245 |
| bwg | 2 | c | 0.007 | 2.286 | 2.7878 |
| bwg | 3 | a | 0.0161 | 3.556 | 2.4873 |
| bwg | 3 | b | 0.0093 | 4.826 | 3.2317 |
| bwg | 3 | c | 0.0177 | 3.048 | 3.9637 |
| lbs | 1 | a | 0.0152 | 5.842 | 4.355 |
| lbs | 1 | b | 0.0101 | 6.858 | 2.2177 |
| lbs | 1 | c | 0.006 | 8.89 | 3.1991 |
| lbs | 2 | a | 0.0042 | 8.89 | 2.6839 |
| lbs | 2 | b | 0.0126 | 6.604 | 2.0621 |
| lbs | 2 | c | 0.0072 | 10.668 | 1.0062 |
| lbs | 3 | a | 0.003 | 6.096 | 3.6699 |
| lbs | 3 | b | 0.0122 | 6.604 | 3.1143 |
| ppc | 2 | c | 0.0008 | 11.176 | 0.3901 |
| ppc | 3 | a | 0.0004 | 8.636 | 0.3608 |
| ppc | 3 | b | 0.0016 | 10.922 | 0.4435 |
| ppc | 3 | c | 0.0015 | 10.16 | 0.1931 |
| sog | 1 | a | 0.043 | 9.652 | 7.989 |
| sog | 1 | b | 0.0273 | 6.35 | 4.3293 |
| sog | 1 | c | 0.056 | 7.62 | 12.4708 |
| sog | 2 | a | 0.017 | 5.334 | 4.0464 |
| sog | 2 | b | 0.0379 | 6.604 | 8.0143 |
| sog | 2 | c | 0.0155 | 4.572 | 4.1617 |
| sog | 3 | a | 0.0106 | 7.112 | 2.1602 |
| sog | 3 | b | 0.0204 | 5.08 | 5.9676 |
| sog | 3 | c | 0.0068 | 6.35 | 1.3533 |

| Species | Plant | Leaf Number | Weight (g) | Thickness (mm) | Projected Leaf Area ($cm^2$) |
|---|---|---|---|---|---|
| b | 1 | a | 0.0291 | 6.604 | 8.5209 |
| b | 1 | b | 0.0349 | 5.842 | 6.5554 |
| b | 1 | c | 0.0257 | 9.144 | 6.6385 |
| b | 2 | a | 0.0427 | 7.62 | 9.0173 |
| b | 2 | b | 0.013 | 4.826 | 3.3751 |
| b | 2 | c | 0.03 | 6.858 | 5.958 |
| b | 3 | a | 0.0233 | 5.588 | 4.9617 |
| b | 3 | b | 0.0313 | 5.08 | 7.8998 |
| b | 3 | c | 0.0233 | 6.096 | 5.3548 |
| bwg | 1 | a | 0.0216 | 4.572 | 4.9788 |
| bwg | 1 | b | 0.0106 | 3.81 | 3.1717 |
| bwg | 1 | c | 0.0053 | 8.636 | 1.4413 |
| bwg | 2 | a | 0.0156 | 1.778 | 4.9352 |
| bwg | 2 | b | 0.011 | 3.556 | 3.7245 |
| bwg | 2 | c | 0.007 | 2.286 | 2.7878 |
| bwg | 3 | a | 0.0161 | 3.556 | 2.4873 |
| bwg | 3 | b | 0.0093 | 4.826 | 3.2317 |
| bwg | 3 | c | 0.0177 | 3.048 | 3.9637 |
| lbs | 1 | a | 0.0152 | 5.842 | 4.355 |
| lbs | 1 | b | 0.0101 | 6.858 | 2.2177 |
| lbs | 1 | c | 0.006 | 8.89 | 3.1991 |
| lbs | 2 | a | 0.0042 | 8.89 | 2.6839 |
| lbs | 2 | b | 0.0126 | 6.604 | 2.0621 |
| lbs | 2 | c | 0.0072 | 10.668 | 1.0062 |
| lbs | 3 | a | 0.003 | 6.096 | 3.6699 |
| lbs | 3 | b | 0.0122 | 6.604 | 3.1143 |
| wpc | 1 | a | 0.0027 | 15.494 | 0.4362 |
| wpc | 1 | b | 0.0029 | 13.716 | 0.4715 |
| wpc | 1 | c | 0.0024 | 10.668 | 0.3535 |
| wpc | 2 | a | 0.003 | 11.684 | 0.6317 |
| wpc | 2 | b | 0.0044 | 14.478 | 0.7762 |
| wpc | 2 | c | 0.002 | 12.446 | 0.3825 |
| wpc | 3 | a | 0.0017 | 8.382 | 0.1962 |
| wpc | 3 | b | 0.0013 | 10.414 | 0.1296 |
| wpc | 3 | c | 0.001 | 8.89 | 0.1143 |
| wwg | 1 | a | 0.0373 | 4.572 | 8.707 |
| wwg | 1 | b | 0.0327 | 4.318 | 7.2717 |
| wwg | 1 | c | 0.0429 | 5.334 | 6.8568 |
| wwg | 2 | a | 0.0672 | 7.874 | 10.7866 |

| Species | Plant | Leaf Number | Weight (g) | Thickness (mm) | Projected Leaf Area ($cm^2$) |
|---|---|---|---|---|---|
| b | 1 | a | 0.0291 | 6.604 | 8.5209 |
| b | 1 | b | 0.0349 | 5.842 | 6.5554 |
| b | 1 | c | 0.0257 | 9.144 | 6.6385 |
| b | 2 | a | 0.0427 | 7.62 | 9.0173 |
| b | 2 | b | 0.013 | 4.826 | 3.3751 |
| b | 2 | c | 0.03 | 6.858 | 5.958 |
| b | 3 | a | 0.0233 | 5.588 | 4.9617 |
| b | 3 | b | 0.0313 | 5.08 | 7.8998 |
| b | 3 | c | 0.0233 | 6.096 | 5.3548 |
| bwg | 1 | a | 0.0216 | 4.572 | 4.9788 |
| bwg | 1 | b | 0.0106 | 3.81 | 3.1717 |
| bwg | 1 | c | 0.0053 | 8.636 | 1.4413 |
| bwg | 2 | a | 0.0156 | 1.778 | 4.9352 |
| bwg | 2 | b | 0.011 | 3.556 | 3.7245 |
| bwg | 2 | c | 0.007 | 2.286 | 2.7878 |
| bwg | 3 | a | 0.0161 | 3.556 | 2.4873 |
| bwg | 3 | b | 0.0093 | 4.826 | 3.2317 |
| bwg | 3 | c | 0.0177 | 3.048 | 3.9637 |
| lbs | 1 | a | 0.0152 | 5.842 | 4.355 |
| lbs | 1 | b | 0.0101 | 6.858 | 2.2177 |
| lbs | 1 | c | 0.006 | 8.89 | 3.1991 |
| lbs | 2 | a | 0.0042 | 8.89 | 2.6839 |
| lbs | 2 | b | 0.0126 | 6.604 | 2.0621 |
| lbs | 2 | c | 0.0072 | 10.668 | 1.0062 |
| lbs | 3 | a | 0.003 | 6.096 | 3.6699 |
| lbs | 3 | b | 0.0122 | 6.604 | 3.1143 |
| wwg | 2 | b | 0.0424 | 7.874 | 6.9657 |
| wwg | 2 | c | 0.0617 | 7.874 | 10.4165 |
| wwg | 3 | a | 0.0887 | 8.89 | 14.779 |
| wwg | 3 | b | 0.1151 | 4.826 | 19.2153 |
| wwg | 3 | c | 0.0782 | 7.112 | 14.2879 |

**Table 6.2.** Root measurements from subsamples of fine roots from three plants of each species at day 55, scanned at 800 Dpi; all plants grown in greenhouse, destructively sampled. B, nodding brome, *Bromus anomalus*; BWG, blue bunch wheatgrass, *Pseudoregneria spicata*; LBS, little blue stem, *Schizachyrium scoparium*; PPC, purple prairie clover, *Dalea purpurea*; SOG, side oats grama, *Bouteloua curtipendula*; WPC, white prairie clover, *Dalea candida*; WWG, western wheatgrass, *Pascopyrum smithii*.

| Species | Plant | Weight (g) | Length (cm) | Projected Area ($cm^2$) | Surface Area ($cm^2$) | Average Diameter (mm) | Length/ Volume ($cm/m^3$) | Root Volume ($cm^3$) | Number of Tips | Forks |
|---|---|---|---|---|---|---|---|---|---|---|
| b | 1 | 0.0324 | 1405.966 | 20.7454 | 65.1736 | 0.1476 | 1405.966 | 0.24 | 12707 | 16401 |
| b | 2 | 0.0294 | 882.4851 | 15.4418 | 48.5117 | 0.175 | 882.4851 | 0.212 | 13856 | 12832 |
| b | 3 | 0.0531 | 2314.747 | 38.2705 | 120.2304 | 0.1653 | 2314.747 | 0.497 | 29751 | 35665 |
| bwg | 1 | 0.0347 | 708.3077 | 12.8471 | 40.3603 | 0.1814 | 708.3077 | 0.183 | 4907 | 6314 |
| bwg | 2 | 0.073 | 421.0314 | 8.5127 | 26.7433 | 0.2022 | 421.0314 | 0.135 | 5098 | 7114 |
| bwg | 3 | 0.108 | 1580.207 | 36.9197 | 115.9865 | 0.2336 | 1580.207 | 0.677 | 18744 | 30378 |
| lbs | 1 | 0.0158 | 531.4423 | 7.8806 | 24.7576 | 0.1483 | 531.4423 | 0.092 | 1541 | 2520 |
| lbs | 2 | 0.0226 | 500.3626 | 8.2375 | 25.879 | 0.1646 | 500.3626 | 0.107 | 1391 | 2574 |
| lbs | 3 | 0.0162 | 248.2791 | 5.3978 | 16.9578 | 0.2174 | 248.2791 | 0.092 | 491 | 897 |
| ppc | 1 | 0.048 | 808.6092 | 20.2005 | 63.4616 | 0.2498 | 808.6092 | 0.396 | 10955 | 16892 |
| ppc | 2 | 0.0296 | 420.5797 | 11.1184 | 34.9295 | 0.2644 | 420.5797 | 0.231 | 4867 | 8484 |
| ppc | 3 | 0.0121 | 129.5923 | 3.1844 | 10.004 | 0.2457 | 129.5923 | 0.061 | 732 | 1044 |
| sog | 1 | 0.0349 | 323.542 | 6.9718 | 21.9026 | 0.2155 | 323.542 | 0.118 | 670 | 1299 |
| sog | 2 | 0.0292 | 792.6469 | 11.4449 | 35.9552 | 0.1444 | 792.6469 | 0.13 | 2182 | 4464 |
| sog | 3 | 0.0076 | 610.2967 | 4.5004 | 14.1383 | 0.0737 | 610.2967 | 0.026 | 3941 | 3374 |
| wpc | 1 | 0.0153 | 169.9138 | 4.7142 | 14.8101 | 0.2774 | 169.9138 | 0.103 | 1795 | 2754 |
| wpc | 2 | 0.0071 | 148.7127 | 3.7296 | 11.7168 | 0.2508 | 148.7127 | 0.073 | 463 | 590 |
| wpc | 3 | 0.0016 | 27.998 | 0.9235 | 2.9014 | 0.3299 | 27.998 | 0.024 | 214 | 448 |
| wwg | 1 | 0.1182 | 2114.213 | 47.9757 | 150.7201 | 0.2269 | 2114.213 | 0.855 | 30623 | 52769 |
| wwg | 2 | 0.0267 | 1297.502 | 19.3356 | 60.7447 | 0.149 | 1297.502 | 0.226 | 12833 | 16000 |
| wwg | 3 | 0.0524 | 726.4842 | 17.0389 | 53.5292 | 0.2345 | 726.4842 | 0.314 | 9392 | 15653 |

**Table 6.3.** Leaf measurements of three mature leaves from at least three plants of each species from raw data at age day 100, scanned at 400 dpi; all plants grown in greenhouse, destructively sampled. B, nodding brome, *Bromus anomalus*; BWG, blue bunch wheatgrass, *Pseudoregneria spicata*; LBS, little blue stem, *Schizachyrium scoparium*; PPC, purple prairie clover, *Dalea purpurea*; SOG, side oats grama, *Bouteloua curtipendula*; WPC, white prairie clover, *Dalea candida*; WWG, western wheatgrass, *Pascopyrum smithii*.

| Species | Plant | Leaf Number | Weight (g) | Thickness (mm) | Leaf Area ($cm^2$) |
|---|---|---|---|---|---|
| b | 1 | a | 0.0336 | 4.318 | 8.7739 |
| b | 1 | b | 0.048 | 4.318 | 7.8459 |
| b | 1 | c | 0.0414 | 6.35 | 7.5308 |
| b | 2 | a | 0.0736 | 4.064 | 14.6142 |
| b | 2 | b | 0.0624 | 4.318 | 12.9673 |
| b | 2 | c | 0.0422 | 5.334 | 9.0527 |
| b | 3 | a | 0.076 | 4.572 | 14.2233 |
| b | 3 | b | 0.0898 | 5.588 | 12.4293 |
| b | 3 | c | 0.0483 | 4.064 | 9.513 |
| bwg | 1 | a | 0.0184 | 4.064 | 2.9478 |
| bwg | 1 | b | 0.0308 | 3.81 | 1.093 |
| bwg | 1 | c | 0.0198 | 3.81 | 1.4959 |
| bwg | 2 | a | 0.0159 | 2.032 | 1.5525 |
| bwg | 2 | b | 0.0208 | 3.302 | 2.7837 |
| bwg | 2 | c | 0.0177 | 3.048 | 2.0681 |
| bwg | 3 | a | 0.0696 | 4.064 | 10.4917 |
| bwg | 3 | b | 0.0408 | 3.81 | 3.7379 |
| bwg | 3 | c | 0.0406 | 3.556 | 3.0704 |
| bwg | 4 | a | 0.0475 | 3.556 | 3.2393 |
| bwg | 4 | b | 0.0355 | 4.318 | 3.0979 |
| bwg | 4 | c | 0.0299 | 3.048 | 3.0333 |
| lbs | 1 | a | 0.0406 | 3.302 | 4.9228 |
| lbs | 1 | b | 0.0773 | 2.54 | 9.4689 |
| lbs | 1 | c | 0.0487 | 2.286 | 6.0084 |
| lbs | 2 | a | 0.0303 | 3.302 | 7.3953 |
| lbs | 2 | b | 0.0546 | 3.048 | 3.6613 |
| lbs | 2 | c | 0.0478 | 3.048 | 6.5366 |
| lbs | 3 | a | 0.0276 | 3.302 | 3.7315 |
| lbs | 3 | b | 0.0362 | 2.54 | 3.6549 |
| lbs | 3 | c | 0.0264 | 2.794 | 4.8853 |
| lbs | 4 | a | 0.0217 | 2.794 | 4.2974 |
| lbs | 4 | b | 0.0405 | 2.54 | 3.5518 |
| lbs | 4 | c | 0.0398 | 2.032 | 6.1875 |
| ppc | 1 | a | 0.067 | 4.318 | 0.2674 |
| ppc | 1 | b | 0.0067 | 3.556 | 0.3835 |
| ppc | 1 | c | 0.0061 | 2.286 | 0.4766 |
| ppc | 2 | a | 0.002 | 3.048 | 0.3571 |

| Species | Plant | Leaf Number | Weight (g) | Thickness (mm) | Leaf Area ($cm^2$) |
|---|---|---|---|---|---|
| ppc | 2 | b | 0.0035 | 4.064 | 0.462 |
| ppc | 2 | c | 0.0042 | 3.556 | 0.1445 |
| ppc | 3 | a | 0.0025 | 3.048 | 0.3093 |
| ppc | 3 | b | 0.0032 | 4.572 | 0.4726 |
| ppc | 3 | c | 0.0044 | 6.35 | 0.1998 |
| ppc | 4 | a | 0.0029 | 5.08 | 0.2509 |
| ppc | 4 | b | 0.0028 | 5.588 | 0.2226 |
| ppc | 4 | c | 0.0038 | 6.096 | 0.3787 |
| sog | 1 | a | 0.0708 | 4.064 | 6.8361 |
| sog | 1 | b | 0.0641 | 4.572 | 8.8654 |
| sog | 1 | c | 0.1002 | 4.064 | 10.0087 |
| sog | 2 | a | 0.1067 | 4.318 | 9.9815 |
| sog | 2 | b | 0.0818 | 3.81 | 10.8041 |
| sog | 2 | c | 0.0198 | 2.54 | 3.0092 |
| sog | 3 | a | 0.0386 | 3.048 | 6.2093 |
| sog | 3 | b | 0.0484 | 3.81 | 7.0094 |
| sog | 3 | c | 0.0409 | 3.556 | 5.9064 |
| sog | 4 | a | 0.0688 | 3.81 | 9.8163 |
| sog | 4 | b | 0.0682 | 4.064 | 7.3054 |
| sog | 4 | c | 0.0678 | 4.318 | 8.7791 |
| wpc | 1 | a | 0.0065 | 6.096 | 0.5204 |
| wpc | 1 | b | 0.0096 | 6.096 | 1.0813 |
| wpc | 1 | c | 0.0082 | 5.588 | 0.6906 |
| wpc | 2 | a | 0.0063 | 5.588 | 0.5171 |
| wpc | 2 | b | 0.009 | 6.096 | 0.5797 |
| wpc | 2 | c | 0.0094 | 6.096 | 0.5596 |
| wpc | 3 | a | 0.0071 | 5.588 | 0.517 |
| wpc | 3 | b | 0.0067 | 5.842 | 0.4029 |
| wpc | 3 | c | 0.0053 | 5.334 | 0.5403 |
| wpc | 4 | a | 0.0034 | 3.81 | 0.2714 |
| wpc | 4 | b | 0.0024 | 3.302 | 0.3256 |
| wpc | 4 | c | 0.0058 | 3.556 | 0.4718 |
| wwg | 1 | a | 0.1515 | 8.89 | 16.7307 |
| wwg | 1 | b | 0.1586 | 9.144 | 17.9702 |
| wwg | 1 | c | 0.1177 | 8.382 | 12.1962 |
| wwg | 2 | a | 0.0886 | 7.874 | 13.2579 |
| wwg | 2 | b | 0.0676 | 8.382 | 15.7756 |
| wwg | 2 | c | 0.1251 | 6.35 | 11.3072 |
| wwg | 3 | a | 0.0569 | 6.858 | 5.8241 |
| wwg | 3 | b | 0.114 | 8.636 | 13.5867 |
| wwg | 3 | c | 0.1457 | 9.652 | 15.8202 |
| wwg | 4 | a | 0.0589 | 7.112 | 7.5633 |
| wwg | 4 | b | 0.0758 | 7.112 | 7.424 |
| wwg | 4 | c | 0.0854 | 6.604 | 8.5468 |

**Table 6.4.** Root measurements from subsamples of fine roots of at least three plants of each species at day 100, scanned at 800 dpi; all plants grown in greenhouse, destructively sampled.. B, nodding brome, *Bromus anomalus*; BWG, blue bunch wheatgrass, *Pseudoregneria spicata*; LBS, little blue stem, *Schizachyrium scoparium*; PPC, purple prairie clover, *Dalea purpurea*; SOG, side oats grama, *Bouteloua curtipendula*; WPC, white prairie clover, *Dalea candida*; WWG, western wheatgrass, *Pascopyrum smithii*.

| Species | Plant | Weight (g) | Length (cm) | Project Area ($cm^2$) | Surface Area ($cm^2$) | Average Diameter (mm) | Length/ Volume ($cm/m^3$) | Root Volume ($cm^3$) | Tips | Forks |
|---|---|---|---|---|---|---|---|---|---|---|
| b | 1 | 0.0096 | 178.5658 | 3.7016 | 11.6291 | 0.2073 | 178.5658 | 0.06 | 2298 | 5685 |
| b | 2 | 0.0127 | 138.727 | 3.3114 | 10.4032 | 0.2387 | 138.727 | 0.062 | 1912 | 3717 |
| b | 3 | 0.027 | 306.0623 | 6.8454 | 21.5054 | 0.2237 | 306.0623 | 0.12 | 4160 | 7053 |
| bwg | 1 | 0.0316 | 264.186 | 7.1181 | 22.3622 | 0.2694 | 264.186 | 0.151 | 3796 | 5016 |
| bwg | 2 | 0.0184 | 124.2067 | 3.453 | 10.848 | 0.278 | 124.2067 | 0.075 | 1932 | 3215 |
| bwg | 3 | 0.0249 | 135.9368 | 3.7477 | 11.7739 | 0.2757 | 135.9368 | 0.081 | 1912 | 1923 |
| bwg | 4 | 0.0142 | 160.368 | 3.5878 | 11.2714 | 0.2237 | 160.368 | 0.063 | 1613 | 1772 |
| lbs | 1 | 0.0299 | 140.8421 | 4.4682 | 14.0374 | 0.3173 | 140.8421 | 0.111 | 1599 | 1593 |
| lbs | 2 | 0.0253 | 381.7717 | 6.6725 | 20.9622 | 0.1748 | 381.7717 | 0.092 | 2005 | 3063 |
| lbs | 3 | 0.0205 | 114.3923 | 2.7132 | 8.5238 | 0.2372 | 114.3923 | 0.051 | 679 | 1135 |
| lbs | 4 | 0.0202 | 95.9146 | 2.5648 | 8.0577 | 0.2674 | 95.9146 | 0.054 | 553 | 939 |
| ppc | 1 | 0.0068 | 40.434 | 1.2327 | 3.8725 | 0.3049 | 40.434 | 0.03 | 377 | 395 |
| ppc | 2 | 0.0184 | 147.582 | 4.1679 | 13.0939 | 0.2824 | 147.582 | 0.092 | 1601 | 1788 |
| ppc | 3 | 0.0048 | 121.9351 | 3.597 | 11.3003 | 0.295 | 121.9351 | 0.083 | 1098 | 1658 |
| ppc | 4 | 0.0172 | 318.1856 | 8.72 | 27.3946 | 0.2741 | 318.1856 | 0.188 | 3190 | 4825 |
| sog | 1 | 0.0143 | 132.8254 | 2.5758 | 8.0922 | 0.1939 | 132.8254 | 0.039 | 836 | 1143 |
| sog | 2 | 0.0229 | 264.5936 | 4.5918 | 14.4257 | 0.1735 | 264.5936 | 0.063 | 1763 | 2136 |
| sog | 3 | 0.0117 | 129.7734 | 2.6644 | 8.3704 | 0.2053 | 129.7734 | 0.043 | 592 | 791 |
| sog | 4 | 0.0175 | 201.4306 | 4.1185 | 12.9387 | 0.2045 | 201.4306 | 0.066 | 1143 | 1096 |
| wpc | 1 | 0.0294 | 215.7305 | 7.8469 | 24.6518 | 0.3637 | 215.7305 | 0.224 | 1374 | 2114 |
| wpc | 2 | 0.0096 | 60.7901 | 1.7327 | 5.4433 | 0.285 | 60.7901 | 0.039 | 582 | 1442 |
| wpc | 3 | 0.0072 | 32.615 | 1.2432 | 3.9057 | 0.3812 | 32.615 | 0.037 | 219 | 412 |
| wpc | 4 | 0.0081 | 72.0839 | 1.9995 | 6.2816 | 0.2774 | 72.0839 | 0.044 | 630 | 1429 |

| Species | Plant | Weight (g) | Length (cm) | Project Area ($cm^2$) | Surface Area ($cm^2$) | Average Diameter (mm) | Length/ Volume ($cm/m^3$) | Root Volume ($cm^3$) | Tips | Forks |
|---|---|---|---|---|---|---|---|---|---|---|
| wwg | 1 | 0.0288 | 194.9709 | 5.6895 | 17.8742 | 0.2918 | 194.9709 | 0.13 | 2852 | 5348 |
| wwg | 2 | 0.0394 | 209.9345 | 7.2705 | 22.841 | 0.3463 | 209.9345 | 0.198 | 3332 | 7146 |
| wwg | 3 | 0.0235 | 155.0254 | 4.6135 | 14.4939 | 0.2976 | 155.0254 | 0.108 | 2283 | 5114 |
| wwg | 4 | 0.0202 | 146.9786 | 4.2172 | 13.2489 | 0.2869 | 146.9786 | 0.095 | 2239 | 4528 |

## 6.3 Model descriptions

**Table 6.5.** Description of variables included in the general linear models predicting the productivity of complex communities of 7 species including: B, nodding brome, *Bromus anomalus*; BWG, blue bunch wheatgrass, *Pseudoregneria spicata*; LBS, little blue stem, *Schizachyrium scoparium*; PPC, purple prairie clover, *Dalea purpurea*; SOG, side oats grama, *Bouteloua curtipendula*; WPC, white prairie clover, *Dalea candida*; WWG, western wheatgrass, *Pascopyrum smithii*; $C_3$, cool season grasses; $C_4$, warm season grasses.

| VARIABLE | DESCRIPTION |
|---|---|
| Productivity | *all models)* the log transformed weight of aboveground plant biomass for pot communities. (Response variable) |
| Species richness | *model a)* The number of species present in communities. (Observed continuous integer variable; 1-7) |
| Species evenness | *model b)* Evar (see Smith and Wilson 1996), calculated to be proportional to the number of individuals present in communities. (Observed continuous variable; 0-1) |
| Functional group richness | *model c)* the number of functional groups present in communities. (Observed continuous integer variable; 1-3) |
| B, BWG, LBS, PPC, SOG, WPC, WWG | *model d)* the number of individuals of each species in the communities. (Observed continuous integer variable; 0-21) |
| Legume, $C_3$, $C_4$ | *model e)* the number of individuals present for each functional group. (Observed continuous integer variable; 0-21) |
| B, BWG, LBS, PPC, SOG, WPC, WWG | *model f)* species present in communities. (Observed categorical dummy variables; 0, 1) |
| Legume, $C_3$, $C_4$ | *model g)* functional group present in communities. (Observed categorical dummy variables; 0, 1) |
| Early relative growth rate* (ERGR) | *model h)* ERGR = (0.0944*(B) + 0.0815*(BWG) + 0.0069*(LBS) + 0.0017*(PPC) + 0.0215*(SOG) + 0.0016*(WPC) + 0.2171*(WWG)) /Density |
| Late relative growth rate* (LRGR) | *model h)* LRGR = (0.8624*(B) + 0.1985*(BWG) + 0.2545*(LBS) + 0.0795*(PPC) + 0.2135*(SOG) + 0.0378*(WPC) + 0.8298*(WWG)) /Density |
| Total relative growth rate* (TRGR) | *model i)* TRGR = (0.4400*(B) +0.1342*(BWG) + 0.1183*(LBS) + 0.0367*(PPC) + 0.1079*(SOG) + 0.0179*(WPC) + 0.4928*(WWG)) /Density |

| VARIABLE | DESCRIPTION |
|---|---|
| Early specific leaf area* (ESLA) | *model j)* ESLA= (230.0888*(B) + 269.0193*(BWG) + 273.9366*(LBS) + 324.1858*(PPC) + 215.3203*(SOG) + 163.1636*(WPC) + 175.3559*(WWG)) /Density |
| Late specific leaf area* (LSLA) | *model j)* LSLA=(188.1436*(B) + 99.6940*(BWG) + 130.8275*(LBS) + 35.9762*(PPC) + 121.8025*(SOG) + 81.2760*(WPC) + 117.1961*(WWG)) /Density |
| Early specific root length* (ESRL) | *model k)* MSRL = (40062.6493*(B) + 12561.6430*(BWG) + 23444.7619*(LBS) + 15148.0624*(PPC) + 24079.2971*(SOG) + 14442.6875*(WPC) + 20974.1465*(WWG)) /Density |
| Late specific root length* (LSRL) | *model k)* LSRL = (12644.1197*(B) + 7684.5960*(BWG) + 7642.5516*(LBS) + 13307.9809*(PPC) + 10973.2380*(SOG) + 7020.6169*(WPC) + 6317.3315*(WWG)) /Density |
| Competitive response* (CR) | *model l)* CR = (10.55*(B) + 13.14*(BWG) + 3.98*(LBS) + 10.84*(PPC) + 15.47*(SOG) + 10.48*(WPC) + 18.24*(WWG)) /Density |
| Competitive effect* (CE) | *model l)* CE = (9.13*(B) + 7.65*(BWG) + 17.81*(LBS) + 15.36*(PPC)+ 12.07*(SOG) + 14.34*(WPC) + 6.34*(WWG)) /Density |

* Species specific data were extrapolated to the community level by multiplying the proportional abundance of each species in the community by the value of the trait for that species, and summing across species to obtain an overall value of that trait for the community. (Observed continuous variables).

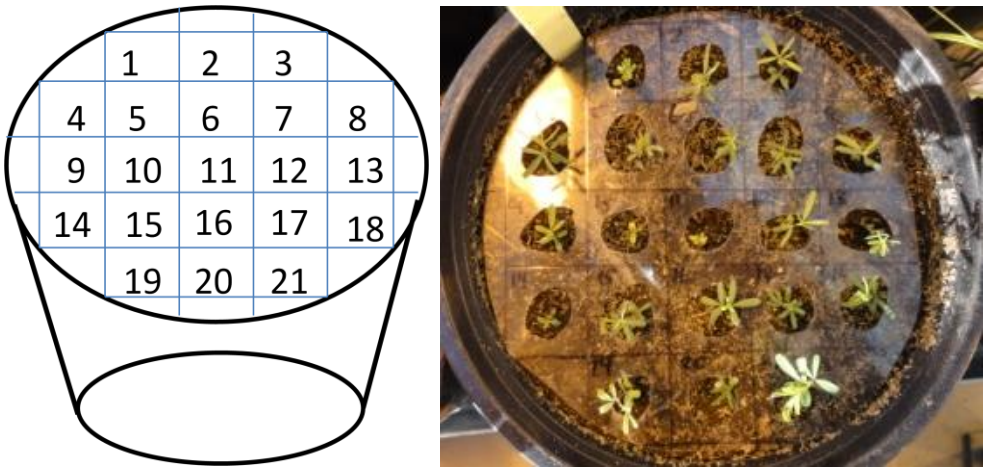


**Figure 6.2.** Planting positions in pot where plants were randomly assigned a planting location within pot.

**Table 6.6.** Summary of direct effects for each of the seven models. B, nodding brome, *Bromus anomalus*; BWG, blue bunch wheatgrass, *Pseudoregneria spicata*; LBS, little blue stem, *Schizachyrium scoparium*; PPC, purple prairie clover, *Dalea purpurea*; SOG, side oats grama, *Bouteloua curtipendula*; WPC, white prairie clover, *Dalea candida*; WWG, western wheatgrass, *Pascopyrum smithii*; $C_3$, cool season grasses; $C_4$, warm season grasses.

| Model | Parameter | Unstandardized Estimate | SE | C.R. | p-value | Standardized Estimate |
|---|---|---|---|---|---|---|
| A | Species Richness | 0.045 | 0.009 | 5.160 | <0.001 | 0.283 |
| B | Evenness | 0.004 | 0.097 | 0.039 | 0.969 | 0.002 |
| C | Functional Group Richness | 0.126 | 0.024 | 0.024 | <0.001 | 0.282 |
| D | SOG | 0.011 | 0.012 | 0.873 | 0.383 | 0.159 |
| | PPC | 0.011 | 0.012 | 0.969 | 0.333 | 0.171 |
| | WWG | 0.025 | 0.011 | 2.209 | 0.027 | 0.372 |
| | WPC | 0.007 | 0.012 | 0.592 | 0.554 | 0.095 |
| | LBS | 0.002 | 0.011 | 0.151 | 0.880 | 0.022 |
| | BWG | 0.021 | 0.021 | 1.791 | 0.073 | 0.274 |
| | B | 0.022 | 0.022 | 1.860 | 0.063 | 0.273 |
| E | Legume | 0.007 | 0.011 | 0.645 | 0.519 | 0.131 |
| | $C_3$ | 0.021 | 0.011 | 1.866 | 0.062 | 0.392 |
| | $C_4$ | 0.005 | 0.011 | 0.422 | 0.673 | 0.082 |
| F | SOG | 0.009 | 0.029 | 0.318 | 0.750 | 0.017 |
| | PPC | 0.015 | 0.03 | 0.512 | 0.609 | 0.028 |
| | WWG | 0.093 | 0.032 | 2.945 | 0.003 | 0.165 |
| | WPC | -0.011 | 0.031 | -0.368 | 0.713 | -0.02 |
| | LBS | -0.002 | 0.032 | -0.077 | 0.939 | -0.004 |
| | BWG | 0.129 | 0.031 | 4.222 | <0.001 | 0.237 |
| | B | 0.056 | 0.031 | 1.822 | 0.068 | 0.103 |
| G | Legume | 0.038 | 0.041 | 0.041 | 0.362 | 0.048 |
| | $C_3$ | 0.347 | 0.048 | 0.048 | <0.001 | 0.384 |
| | $C_4$ | 0.030 | 0.042 | 0.042 | 0.480 | 0.038 |

**Table 6.7.** Summary of covariances for the models with assigned covariances. B, nodding brome, *Bromus anomalus*; BWG, blue bunch wheatgrass, *Pseudoregneria spicata*; LBS, little blue stem, *Schizachyrium scoparium*; PPC, purple prairie clover, *Dalea purpurea*; SOG, side oats grama, *Bouteloua curtipendula*; WPC, white prairie clover, *Dalea candida*; WWG, western wheatgrass, *Pascopyrum smithii*; $C_3$, cool season grasses; $C_4$, warm season grasses.

| Model | Parameter | Estimate | SE | C. R. | p-value | Standardized Estimate |
|---|---|---|---|---|---|---|
| D | B<-->BWG | -1.088 | 0.624 | -1.744 | 0.081 | -0.1 |
| | B<-->LBS | -0.984 | 0.61 | -1.614 | 0.107 | -0.093 |
| | B<-->PPC | -2.318 | 0.744 | -3.115 | 0.002 | -0.181 |
| | B<-->SOG | -1.929 | 0.733 | -2.632 | 0.008 | -0.152 |
| | B<-->WPC | -2.027 | 0.651 | -3.113 | 0.002 | -0.181 |
| | B<-->WWG | -1.797 | 0.722 | -2.490 | 0.013 | -0.144 |
| | BWG<-->LBS | -0.919 | 0.637 | -1.443 | 0.149 | -0.083 |
| | BWG<-->PPC | -2.256 | 0.777 | -2.906 | 0.004 | -0.168 |
| | BWG<-->SOG | -2.547 | 0.772 | -3.301 | <0.001 | -0.192 |
| | BWG<-->WPC | -1.276 | 0.674 | -1.893 | 0.058 | -0.109 |
| | BWG<-->WWG | -3.112 | 0.768 | -4.053 | <0.001 | -0.238 |
| | LBS<-->PPC | -1.514 | 0.754 | -2.008 | 0.045 | -0.116 |
| | LBS<-->SOG | -3.046 | 0.761 | -4.002 | <0.001 | -0.235 |
| | LBS<-->WPC | -2.373 | 0.669 | -3.547 | <0.001 | -0.207 |
| | LBS<-->WWG | -1.177 | 0.733 | -1.605 | 0.108 | -0.092 |
| | PPC<-->SOG | -3.885 | 0.921 | -4.220 | <0.001 | -0.249 |
| | PPC<-->WPC | -2.974 | 0.808 | -3.680 | <0.001 | -0.215 |
| | PPC<-->WWG | -2.451 | 0.892 | -2.748 | 0.006 | -0.159 |
| | SOG<-->WPC | -1.323 | 0.785 | -1.684 | 0.092 | -0.097 |
| | SOG<-->WWG | -3.301 | 0.892 | -3.702 | <0.001 | -0.217 |
| | WPC<-->WWG | -2.35* | 0.782 | -3.004 | 0.003 | -0.174 |
| E | legume<-->$C_3$ | -12.679 | 1.517 | -8.356 | <0.001 | -0.544 |
| | legume<-->$C_4$ | -9.095 | 1.312 | -6.933 | <0.001 | -0.432 |
| | $C_3$<-->$C_4$ | -10.857 | 1.423 | -7.632 | <0.001 | -0.485 |
| F | B<-->BWG | 0.048 | 0.014 | 3.539 | <0.001 | 0.207 |
| | B<-->LBS | 0.044 | 0.013 | 3.410 | <0.001 | 0.199 |
| | B<-->PPC | 0.036 | 0.014 | 2.671 | 0.008 | 0.155 |
| | B<-->SOG | 0.012 | 0.013 | 0.908 | 0.364 | 0.052 |
| | B<-->WPC | 0.036 | 0.013 | 2.782 | 0.005 | 0.161 |
| | B<-->WWG | 0.028 | 0.013 | 2.190 | 0.029 | 0.126 |

| Model | Parameter | Estimate | SE | C. R. | p-value | Standardized Estimate |
|---|---|---|---|---|---|---|
| | BWG<-->LBS | 0.035 | 0.013 | 2.680 | 0.007 | 0.155 |
| | BWG<-->PPC | 0.042 | 0.014 | 3.037 | 0.002 | 0.176 |
| | BWG<-->SOG | 0.018 | 0.014 | 1.292 | 0.196 | 0.074 |
| | BWG<-->WPC | 0.030 | 0.013 | 2.300 | 0.021 | 0.133 |
| | BWG<-->WWG | 0.028 | 0.013 | 2.922 | 0.003 | 0.169 |
| | LBS<-->PPC | 0.026 | 0.013 | 2.020 | 0.043 | 0.116 |
| | LBS<-->SOG | 0.003 | 0.013 | 0.208 | 0.835 | 0.012 |
| | LBS<-->WPC | 0.013 | 0.012 | 1.076 | 0.282 | 0.062 |
| | LBS<-->WWG | 0.042 | 0.013 | 3.293 | <0.001 | 0.192 |
| | PPC<-->SOG | 0.009 | 0.014 | 0.635 | 0.525 | 0.036 |
| | PPC<-->WPC | 0.008 | 0.013 | 0.621 | 0.534 | 0.036 |
| | PPC<-->WWG | 0.036 | 0.013 | 2.750 | 0.006 | 0.159 |
| | SOG<-->WPC | 0.030 | 0.013 | 2.298 | 0.022 | 0.133 |
| | SOG<-->WWG | 0.029 | 0.013 | 2.191 | 0.028 | 0.126 |
| | WPC<-->WWG | 0.000 | 0.012 | 0.027 | 0.978 | 0.002 |
| G | legume<-->$C_3$ | 0.001 | 0.006 | 0.132 | 0.895 | 0.008 |
| | legume<-->$C_4$ | 0.003 | 0.006 | 0.472 | 0.637 | 0.027 |
| | $C_3$<-->$C_4$ | 0.017 | 0.006 | 3.066 | 0.002 | 0.178 |

## 6.4 References


**Cornelissen, J. H. C., Lavorel, S., Garnier, E., Díaz, S., Buchmann, N., Gurvich, D. E., Reich, P. B., Steege, H. t., Morgan, H. D., Heijden, M. G. A. v. d., Pausas, J. G. and Poorter, H. 2003**. A handbook of protocols for standardised and easy measurement of plant functional traits worldwide. Aust. J. Bot. **51**: 335-380.

**Levang-Brilz, N. and Biondini, M. E. 2003**. Growth rate, root development and nutrient uptake of 55 plant species from the Great Plains Grasslands, USA. Plant Ecol. **165**: 117-144.